IMPACTS OF DIRECT-FED MICROBIAL SUPPLEMENTATION ON THE ACUTE IMMUNE RESPONSE AND STATISTICAL PROCESS CONTROL ALGORITHMS TO DETECT BOVINE RESPIRATORY DISEASE

A Dissertation

by

WILLIAM CHRISTIAN KAYSER

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Chair of Committee,	Gordon Carstens
Co-Chair of Committee,	William Pinchak
Committee Members,	Amarnath Banerjee
	Kevin Washburn
Head of Department,	G. Cliff Lamb

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ABSTRACT

Two studies were conducted to examine the impact of dietary supplementation with *Saccharomyces cerevisiae boulardii* (LY) on immunological, physiological and behavioral responses to experimental *Mannheimia haemolytica* (MH) and viral-bacterial (VB) challenges in beef cattle. In both studies, the MH and VB challenges impacted leukogram constituents, animal behavior, and body temperature responses; consistent with an acute immune response. In the MH-challenge study, LY-supplemented steers exhibited reduced ($P < 0.01$; 1.52 vs. 1.74, kg) ADG and G:F ($P < 0.01$; 0.14 vs. 0.16) compared to control steers during the 28 d prior to challenge. However, during the 28 d post challenge period LY-supplemented steers had improved ADG ($P < 0.01$; 1.50 vs. 1.13, kg) and G:F ($P < 0.01$; 0.15 vs. 0.11) compared to control steers. Furthermore, LY supplementation increased ($P = 0.02$) cortisol 32% throughout the study, but did not impact any other serological measures. In the VB-challenge study, LY-supplemented heifers had greater neutrophil production 16% ($P = 0.02$), increased monocytes ($P < 0.05$) on day 4, and reduced haptoglobin concentration ($P < 0.05$) on day 5 compared to control heifers. These results indicate LY supplementation altered immune response during disease challenge; however, the effects of LY supplementation on growth and performance requires further investigation.

Animal-health monitoring systems that utilize real-time biosensor systems for preclinical disease detection are dependent upon data-processing algorithms that can accurately differentiate between healthy and morbid animals. Objectives of this research

were to develop and evaluate various statistical process control (SPC) algorithms, for use in an animal-health monitoring system. For this objective, the 2 challenge studies and a field-based BRD observational study were used. The field-based BRD observational study consisted of 231 bulls on a feed efficiency test, during which 30 were identified as morbid. The SPC models were developed using 3 types of biosensor data collection systems; including, DMI and feeding behavior, ruminal temperature (RUT) and accelerometer-based behaviors. In the observational study, DMI was the most accurate (80%) of the phenotypic response variables followed by head down (HD) duration (79%), which signaled 4.8 d prior to clinical symptoms. Principal components analysis (PCA) was used to construct multivariate models using 3 feeding behavior traits with and without DMI. The feeding behavior model with DMI was 84% accurate and signaled 2 d prior to clinical symptoms. Removal of DMI did not impact the accuracy, and minimally altered signal day. In the MH-challenge study, accuracies of SPC models for DMI, BV duration and RUT were 89, 89 and 86%, respectively, which signaled 0.14, 0.13 and 0 days after the MH challenge, respectively. In the VB-challenge study, DMI was the most accurate response variable with an accuracy of 95% and signaled the day of MH inoculation, followed by rest, meal duration and RUT (89, 87 and 94%, respectively). The SPC models for DMI, feeding behaviors and RUT were consistently more accurate for monitoring the health status of beef cattle than the accelerometer-based behavior traits. These results illustrate the effectiveness of using SPC procedures coupled with remote data collection sensors for preclinical detection of BRD in beef cattle.

DEDICATION

I would like to dedicate this dissertation to my family Melissa, Izzy and Ace. Without their sacrifice and love this dissertation and my completion of a Ph.D. would have never been realized.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

There is renewed sense of importance in the development of effective animal health strategies that will reduce the reliance on the use of antibiotics in animal agriculture. Public interest and concern of the use of antibiotics within the livestock industry has increased the emphasis placed on disease prevention and use of direct fed microbials (DFM) in lieu of sub-therapeutic antibiotics and ionophores. In beef cattle, bovine respiratory disease (BRD) accounts for 67 to 87% of the morbidity events within feedyards (Edwards, 1996), which is exacerbated when animals are stressed. Early preclinical detection of BRD is difficult and current industry approaches rely on visual appraisal of clinical symptoms (Broom, 2006), which are not always accurate. Current estimates show that visual observation is highly specific (0.92) but not very sensitive (0.27), indicating that many animals contract BRD but are not detected (Timsit et al., 2016).

Recent developments in sensor technologies enable real-time measurements of feeding behavior patterns and feed intake on an individual animal basis (Kayser and Hill, 2013, Lancaster et al., 2009; Theurer et al., 2013), which have been shown to be predictive of morbidity events (Quimby et al., 2001, Sowell et al., 1999). Furthermore, previous studies have also examined the use of other sensors, such as 3-axis accelerometers, triangulation global positioning systems and ruminal bio-thermal

boluses to quantify deviations in animal behavioral and physiologic status relative to the onset of disease (Richeson et al., 2018; Timsit et al., 2016). These biosensor data collection systems coupled with robust mathematical models can enhance the accuracy of these systems for preclinical BRD disease detection. Improvement in detection would improve the efficacy of medical intervention (Cusack, et al., 2003), lead to more judicious application of antimicrobial therapy (Schaefer, et al., 2007) and mitigate the production losses associated with undiagnosed BRD.

Cattle in feedlots are frequently at risk for developing digestive disturbances that result in compromised health and growth performance (Wagner et al., 2016). Growth promoting antimicrobials such as ionophores are commonly used in North American beef production systems and reduce the variation in feed intake while improving feed efficiency. However, there are many countries where these products are not approved for use, and there are high value markets such as natural or organic that exclude the use of ionophores and sub-therapeutic antibiotics. Potential alternatives to sub-therapeutic antibiotics that can produce selective changes in the microbial populations of the gastro intestinal tract are DFM's (NASEM, 2016). Yeast products such as *Saccharomyces cerevisiae* are widely used in commercial ruminant production, specifically dairies because they tend to improve milk yield (Beauchemin et al., 2006). The effects of DFM on growth and production in beef cattle is variable across studies with inconclusive results. Although, when multiple studies were included in a meta-analysis (Wagner et al., 2016) the inclusion of *S. cerevisiae* increased final BW (2.9, kg), ADG (6.5 %), DMI (1 %) and G:F (2.6 %) compared to the controls. Reductions in morbidity and improved

treatment success rates have also been reported due to supplementation of DFM. Therefore, DFM supplementation could potentially serve as a non-antibiotic approach to improving health of newly received or stressed cattle (Duff and Galyean, 2007).

Antibiotic resistance is a major social and political concern in the United States, which has prompted the potential removal of antimicrobials from all feedstuffs (Finck et al., 2014). If this becomes reality, the industry will need to adopt management changes to reduce the incidence of morbidity and developed new treatment strategies. There is a need for an improved method of detecting BRD in feed yard cattle, as well as increased knowledge in the effect and dose of DFM for improving performance and mitigating BRD in feedyard cattle.

Bovine Respiratory Disease

Overview

Bovine respiratory disease has been an active research topic and a challenge to beef cattle producers for over 50 years. There have been few improvements in reducing the incidence of BRD, and mortalities during the feeding period tend to be increasing (Engler et al., 2014). Challenges with vaccination and treatment of the disease complex are in part due to its multi-factorial nature and interaction of stressors, and viral and bacterial pathogens (DeDonder and Apley, 2015). A recent survey was conducted (Terrell at al., 2011) of consulting feedyard veterinarians to identify the health recommendations given to feedyard managers for all areas of animal health management. The veterinarians who were selected, represented clients whose feeding

capacity accounts for approximately 34% of all of the cattle on feed in the United States and Canada. With regards to control of BRD, all of the veterinarians recommended vaccination of bovine herpes virus-1 (BHV-1) upon arrival regardless of risk classification. In high-risk cattle all veterinarians recommended vaccination for bovine viral diarrhea virus (BVDV) types 1 and 2, but that recommendation was reduced to 95.7% for both types when the animals were considered low risk. The respondents were half as likely to recommend vaccination to protect against bovine respiratory syncytial virus (BRSV; high risk = 65.2%, low risk = 52.2%) and parainfluenza-3 virus (PI-3; high risk = 60.9%, low risk = 52.2%) for both classes of cattle. Vaccination usage for the control for the bacterial pathogens within the BRD complex was much lower than the viral components, with the exception of *Mannheimia haemolytica* (MH) where 73.9% of the veterinarians recommended vaccination in high risk cattle but none in low risk cattle. The other bacterial pathogens were less likely to be administered to high risk cattle, *Histophilus somni* (HS), *Pasteurella multocida* (PM) and *Mycoplasma bovis* (MB) were recommended 21.7, 34.8 and 0% in high risk cattle, respectively and between 0 and 5% in low risk cattle. Based upon the results of the survey, cattle within the feedyard should be well prepared to mount an effective immune response to the pathogens within the BRD complex.

Pathogens

The most prevalent pathogen associated with BRD is MH, which is known to cause outbreaks in all ages of cattle (Ackerman and Brogden, 2000). Although

considered a commensal bacteria and often found in the upper respiratory tract of healthy animals, it is also opportunistic and has been found to be the most common isolate from feedlot cattle with fatal fibrinous bronchopneumonia (Smith, 2009). The virulence factors of MH allow the pathogen to successfully evade portions of the innate and adaptive immune system.

Formerly known as *Pasteurella haemolytica*, MH is a gram-negative, weakly hemolytic, facultative aerobic bacterium with at least 12 identified serotypes (Griffin et al., 2010; Highlander, 2001). There are pathogenic and non-pathogenic serotypes, and the pathogenic serotypes are species specific (Smith, 2009). Worldwide, serotype A1 is the most prevalent in cattle and recognized as the most common cause of disease (Highlander, 2001). The organism exists as normal flora of the upper respiratory tract in healthy ruminants, mainly in the nasopharynx and tonsillar crypts (Griffin et al., 2010). If the animal becomes stressed due to viral infections, external stressors, or any event that reduces the functionality of the innate immune system, MH multiplies rapidly in the nasopharynx (Step and Confer, 2009; Highlander, 2001). This is easily measureable with retro-pharyngeal nasal swabs taken pre and post shipping or during a stress event (Frank et al., 2002). Through a mechanism that is poorly understood (Ackerman and Brogden, 2000), the bacteria is able to migrate into and colonize the lung. It is believed that the increase of MH in the nasopharynx allows it to be more easily aspirated into the lung or transferred as a droplet from one animal to another (Highlander, 2001). The MH binds to the epithelial surface and is resistant to physical removal by airflow and mucociliary clearance (Singh et al., 2011). Organisms that are inhaled into the lungs are typically

cleared within hours, but under conditions of impaired pulmonary defenses or innate immune functions, the bound MH is able to proliferate rapidly within the lung (Smith, 2009). Calves that are co-infected with respiratory viruses (BHV-1, PI-3, BVDV, or BRSV) exhibit increased sensitivity to MH infection and subsequently are more susceptible to severe pneumonia (Smith, 2009). Co-infection with BHV-1 is common due to the nature that BHV-1 infects the upper respiratory tract. Rhinotracheitis caused by BHV-1 erodes the epithelium and causes necrosis of cilia and goblets cells. This sequence of events favors colonization and migration of MH into the lower respiratory tract (Srikumaran et al., 2007).

Virulence Factors and Immune Response

After an MH infection becomes established within the lungs, the host's defenses interact with the bacteria, resulting in tissue damage. There are a variety of virulence factors that MH possess which reduce the ability of the immune response to eradicate it and amplify the inflammatory response. These virulence factors include adhesin proteins, polysaccharide capsule, transferrin-binding proteins, various proteases, leukotoxin, and lipopolysaccharide (Singh et al., 2011). Alveolar epithelial cells contain carbohydrate on the surface, and the adhesin proteins on the capsule of MH are thought to improve the ability of colonization by increasing the potential for attachment to theses epithelial cells (Ackerman and Brogden, 2000; Rice et al., 2007). Many bacteria possess polysaccharide capsules which are believed to reduce the phagocytic ability of neutrophils and mononuclear phagocytes (Srikumaran, et al., 2007).The polysaccharide

capsule has been experimentally found to reduce leukocyte phagocytosis and reduce complement mediated lysis (Sing et al., 2011). Chae et al. (1990) compared the serum-mediated immune defense mechanisms effect on encapsulated and decapsulated MH. When the capsular material was removed MH became more susceptible to serum agglutination, complement mediated killing and phagocytosis by neutrophils than intact capsulated MH. The capsular structure is also known to increase migration of neutrophils to the site of infection (Rice et al., 2007). To further evade discovery of the immune system, MH contains various proteases that cleave IgG antibodies and reduce the effectiveness of availability for proliferation within a host. MH contains multiple outer membranous proteins that are physiologically and pathologically relevant because they are involved in iron acquisition (Singh et al., 2011). These membranes contain transferrin-binding protein and other iron regulated proteins that allow MH to replicate in the low iron environment typically maintained by the host (Rice et al., 2007). These factors increase the pathogenicity of MH by increasing the ability for it to attach and colonize the host while reducing the ability for the immune system to discover and eradicate it.

Leukotoxin is an exotoxin that is produced during the logarithmic growth phase of MH and is lethal to ruminant leukocytes (Smith, 2009). It is pivotal to the induction of pneumonia (Rice et al., 2007). The effects of leukotoxin on bovine leukocytes is dose dependent. In low concentrations, leukotoxin activates neutrophils and macrophages to stimulate respiratory burst and degranulation (Rice et al., 2007). This results in the release of proinflammatory cytokines (TNF-α, IL-1, and IL-8) from neutrophils and

alveolar macrophages. Degranulation of the mast cells induces localized histamine release (Singh et al., 2007). Increasing the concentration of leukotoxin stimulates target cells to undergo apoptosis (Rice et al., 2007). At high levels of leukotoxin, bovine plasma membranes form transmembrane pores (Highlander, 2001), which results in efflux of K+ and influx of Ca_2+ (Rice et al., 2007). Subsequently, water moves into the cell to correct the imbalance, resulting in rapid cell swelling (Singh et al., 2007) and ultimately oncotic cell death. These transmembrane pores also cause leakage of respiratory burst particles, such as hydrogen peroxide, oxygen free radicals, and superoxide anions, into the surrounding alveolar parenchyma, which contribute to pulmonary damage (Singh et al., 2007).

Lesion Formation

Acute pulmonary infection is typically characterized by fibrinopurulent and necrotizing inflammatory response. Parenchymal necrosis is most likely a result from infiltration of neutrophils, lipopolysaccharide and leukotoxin (Rice et al., 2007). Neutrophil mediated damage to the lung mucosa removes an innate barrier and allows exposure of capillaries and small vessels to inflammatory mediators released in the lung and bacteria within the exudate (Ackerman and Brogden, 2000). The extent of the tissue damage caused by neutrophils is unclear. Slocombe et al. (1985) experimentally inoculated calves with MH that had normal levels of neutrophils or were neutrophil depleted. Animals were euthanized 6 h post inoculation and a necropsy was performed. The animals with normal levels of neutrophils developed lung lesions that consisted of

necrosis of the alveolar walls, intra-alveolar hemorrhage and a severe exudative and necrotizing bronchopneumonia, with accumulation of proteinaceous fluid in the alveolar lymphatics. In contrast, MH inoculation of neutrophil depleted calves yielded lungs that were grossly normal, leading the authors to conclude that neutrophils were required for acute lung injury. In contrast, Breider et al. (1988) found lesions in both neutrophil sufficient and deficient calves. However, the nature of the lesions were different. Neutrophil-sufficient calves developed fibrinopurulent alveolitis-bronchitis with associated alveolar septal necrosis, interlobular edema and intravascular thrombosis. The neutrophil-deficient calves had extensive intra-alveolar edema, interlobular edema, intra-alveolar hemorrhage, and focal areas of alveolar septal necrosis. These results showed that tissue damage from MH occurs through neutrophil dependent and independent mechanisms (Breider et al., 1988). Although the effect of neutrophils is inconclusive, both studies showed that MH can create severe lung lesions within 6 h of inoculation and that the neutrophils are responsible for fibrinopurulent lesions and accumulation of fibrin in the lungs.

Depending on the size and distribution of these lesions, the infection can result in death. Typical necropsy findings in animals infected with MH are fibrinopurulent bronchopneumonia or pleuropneumonia, primarily in the cranioventral lung (Confer, 2009). However, in severe cases the majority of the lung may be affected. Interlobular septa are expanded with leaked gelatinous proteinaceous fluid that is clear to yellow in color (Smith, 2009). Inflamed areas of the lung may be covered with fibrin and adhered to the pleura of the thoracic wall. Thrombi are frequently found in pulmonary vessels

and are thought to account for infarction and lead to subsequent necrosis (Step and Confer, 2009). The cut surface of the lung often reveals marbling of red and gray, and whole lobules will undergo hemorrhage or coagulative necrosis (Confer, 2009). Animals that survive the acute stage of pneumonia may have multiple abscesses and pleural adhesions (Smith, 2009). In most cases of BRD, the animals are co-infected with multiple viruses and bacteria, although these pathological findings are relatively unique to MH.

The pathogenesis of MH is elaborate, aided by the unique virulence factors that it possesses, which allow the organism to evade the innate and active immune system, resulting in infection. The leukotoxin that MH produces is paramount to its pathogenicity and allow the organism to work synergistically with other bacteria and viruses associated with the BRD complex. Damage to pulmonary tissue results from the inflammatory effects of pro-inflammatory cytokines, leukotoxin, lipopolysaccharide and neutrophils. Even though the majority of cattle are vaccinated for viral and bacterial pathogens within the BRD complex (Terrell et al., 2011), it continues to be the most prevalent and costly disease to the cattle feeding industry.

Objective Disease Detection

Current Detection Methods

One of the challenges with the BRD complex is that it is difficult to detect in cattle during the early stages of an infection. There is widespread agreement that accurate preclinical detection of BRD is crucial for effective intervention of this disease

(Apley, 1997). Identification of morbid cattle earlier in the disease process through objective behavioral monitoring, would potentially improve the efficacy of antimicrobial therapy (Ferran et al., 2011), and therefore reduce mortalities as well as the duration of time animals are in a morbid state. The economic burden associated with BRD is much further reaching than the losses associated with mortality; morbidity is the largest non-feeding cost associated with feeder cattle production (Pinchak, et al., 2004), due to the increases in labor associated with treatment and production losses pre and post illness (Smith, 1998). When animals are morbid they are unproductive (Smith, 2015) because they divert energy from creating products (milk, muscle and fiber) to mount a defensive response to the disease. Many cases of BRD go untreated as diagnosis is difficult and relies on visual appraisal of clinical illness (Broom, 2006). Numerous studies have documented poor to fair associations between observational detection of BRD and prevalence of lung lesions at harvest. In fact, Timsit et al. (2016) reported the sensitivity of BRD detection based on subjective observation of clinical signs was only 27%, which indicates that BRD cases often go undetected, or do not get detected until later in the disease process when successful intervention in less likely (Janzen et al., 1984). Thus, there is a critical need to develop robust animal-health monitoring systems that are more sensitive in detecting BRD in order to improve efficacy of antimicrobial intervention and more specific to limit unnecessary use of antimicrobials, which would ultimately lead to improvement in animal welfare, profitability and perception of the industry.

Behavior Patterns

Altered behavioral patterns associated with consumption of feed and water are among the earliest indicators of the onset of infectious disease. In calves at high-risk for BRD upon feedlot arrival, Daniels et al. (2000) and Sowell et al. (1999) found that calves diagnosed and treated for BRD spent 23 to 42% less time at the feed bunk and had 10 to 36% fewer feeding and drinking bouts compared to untreated calves that did not display clinical symptoms of BRD. Frequency and duration of feeding bouts are known to be positively correlated with feed intake in beef cattle (Lancaster, et al., 2009, Kayser, et al., 2013). Thus, the calves that had BRD in these studies likely consumed less feed as evidenced by their lower daily gains. Carroll and Forsberg (2007) concluded that the increase in energy required to produce pro-inflammatory cytokines, acute phase proteins, antibodies and mount febrile responses to infectious disease creates a state of hyper-metabolism. As such, animals compensate for this increased energy demand by altering various behavioral responses such as increased time for sleep, reductions in social activity, sexual behavior and feed intake in order to conserve energy. Jackson et al. (2016) used a 2-slope broken-line regression model to characterize deviations in DMI and feeding behavior patterns preceding the onset of observed clinical symptoms associated with BRD in cattle. The model-detected breakpoint for DMI occurred 6.8 d prior to observed clinical illness, whereas, breakpoints for BV frequency and duration were 7.6 and 7.2 d prior to observed clinical illness. Lukas et al. (2008) reported pre-clinical reductions of DMI in dairy cows with mastitis and reductions in water intake associated with the febrile response. Using pattern recognition techniques, Moya et al.

(2015) reported that morbid cattle exhibit distinctive deviations in feeding behavior patterns prior to displaying overt clinical symptoms of BRD and can be differentiated from the feeding patterns of healthy cattle. Based on discrete survival time analysis of DMI and feeding behavior data, Wolfger et al., (2015) reported that increases in DMI per meal, meal frequency and inter-meal interval were associated with a decreased hazard for developing BRD up to 7 d prior to observed clinical symptoms of disease. The results from these studies suggest that deviations in feeding behavior patterns preceding the display of clinical symptoms of illness in beef cattle may be useful in development of predictive algorithms for preclinical detection of BRD.

Objective Detection Methods

Few studies have been conducted that have monitored changes in behavioral and/or physiological patterns of individual animals to predict the onset of disease. Other attempts at early identification have used hazard analyses (Wolfger et al., 2015), mean comparison (Sowell, et al., 1999), logistic regression (Schaefer et al., 2007) and cluster analyses (Moya et al., 2015). There are also studies and commercial biosensors available that have published results without clearly defining the method or describing the algorithm that was used to predict BRD. Using a novel high-frequency active integrated electronic system that measured BV frequency and duration MacGregor et al. (2015) monitored the health of high risk calves and reported a reduction in morbidity and an increase in treatment success rate compared to visual observation of clinical illness. White et al. (2015), using the remote early disease identification (REDI) system were in

agreement on morbid animals 94% of the time when compared to visual observation, and were able to identify morbidity 0.75 d prior to visual observation of clinical illness. The predictive algorithms used in the REDI system are proprietary, and therefore cannot be described, although it appears the predictions are made based upon monitoring a suite of behavior measurements.

Statistical process control (SPC) charts were first proposed by Shewhart (1931) to detect abnormal variance within process and were first applied in the manufacturing industries, but applications have also been used in service, financial and health care industries (De Vries and Reneau, 2010, Montgomery, 2009). The SPC procedures have been employed with success in multiple livestock species. Devries and Reneau (2010) reviewed 28 studies that employed SPC procedures to monitor various aspects of livestock production. Of their 28 studies, only 2 were conducted in beef cattle, with the remainder conducted in swine, poultry or dairy production systems. The traits that were monitored in these studies included morbidity, electro-conductivity of milk, muscle pH and conception rates. Furthermore, the majority of the studies were conducted on a herd or pen level rather than on an individual-animal basis. Devries and Reneau (2010) concluded that carefully constructed control charts are powerful methods to monitor animal production systems, and that application of these methods will grow with advancement of biological sensor and computer technologies to monitor individual-animal health status and performance.

Most studies that have examined the value of animal-health monitoring systems have used high-risk cattle. Quimby et al. (2001) monitored cattle that had an overall

morbidity rate of 67%, whereas in other studies morbidity rates ranged from 77% (Wolfger et al., 2015) to 29 % (Moya et al., 2015), which are much higher than typical morbidity rates reported in commercial feedyards (mean morbidity rate = 14%, Irsik, et al., 2006). It is unknown whether using high-risk cattle to develop a predictive model would affect the accuracy of the model to identify morbid animals in "typical" commercial settings. Using high-risk cattle certainly increases the probability of true positives.

Direct-Fed Microbials

Overview

There has been a renewed interest in the use of direct-fed microbials (DFM) in beef production. The US Food and Drug Administration (FDA) defines DFM as "a source of live (viable) naturally occurring microorganisms" (Yoon and Stern, 1995). There are two main types of DFM currently in use, bacterial DFM typically populate in the small intestine and reduce bacterial population or gastro intestinal tract adhesion through competitive exclusion, and yeast cultures which effect the rumen microbial populations. *Saccharomyces cerevisiae* (LY) are widely used in commercial ruminant production (Beuchemin et al., 2006). The goal of utilizing these products is to modify the rumen environment to increase productivity and health of the animal, while reducing the use of direct-fed antibiotics and sub-therapeutic antibiotics. Removal of direct-fed antimicrobials in beef production would allow producers to access markets where those practices are restricted, and more importantly reduce the opportunity for anti-microbial

resistance. Proposed LY modes of action occur in the rumen where it is believed that they favorably alter digestion through modulation of acid production, promotion of desirable microbial populations, enhancement of ruminal fiber digestion and scavenge oxygen within the rumen (McAllister et al., 2011; Newbold et al., 1996).

Effects on Growth and Performance

The effects supplementation of LY in growing beef cattle are inconclusive and there are thought to be interactions between animal type, stress level and percent concentrates of the diet. In recently weaned crossbred steers, Finck et al. (2014) reported, an increase in DMI of 9% over 56 d compared to control calves. However, Keyser et al. (2007) found no differences in production metrics of heifers supplemented with LY compared to controls, although animals that were supplemented with LY exhibited less of a DMI suppression in response to metaphylactic treatment of florfenicol. In a recent meta-analysis conducted by Wagner et al. (2016), 18 experiments conducted on animals being fed growing and finishing diets were identified to evaluate the effects of LY supplementation on feedlot performance and carcass characteristics. Cattle supplemented with LY exhibited increased final BW (2.9 kg), ADG (6.5%), DMI (1%) and G:F (2.6%) relative to the control cattle. Supplementation of LY also improved the percentage of animals grading low choice or higher (LY = 73% vs. CON = 64%) and had a tendency ($P < 0.06$) to reduce liver abscesses (LY = 19% vs. CON = 24%).

Effects on Morbidity and Mortality

Cole et al. (1992) conducted 3 experiments to identify the effects of *Saccharomyces cerevisiae* on health and performance of newly received cattle and cattle experimentally inoculated with BHV-1. The yeast culture did not significantly affect the health or performance of calves in the receiving studies, although morbid cattle in experiment 2 that were fed yeast required fewer days of antibiotic therapy. When steers were challenged with BHV-1, calves that were fed yeast tended to maintain heavier weights and DMI relative to the control animals. Furthermore, Keyser et al. (2007) reported that high risk calves supplemented with yeast had reduced morbidity (13.8 vs. 24.0 %) compared to the controls when both treatments were treated metaphylactically. Although, there were no differences when the animals were not metaphylactically treated or in DMI, ADG and G:F.

Summary and Conclusion

Regardless of the advancements made in pharmaceutical products designed to combat BRD, the disease complex continues to negatively impact animal welfare and profitability. Furthermore, with consumer preferences shifting to natural and organic protein sources the ability for beef cattle producers to utilize antibiotics may be waning. Inclusion of DFM into the diet of cattle during the feeding phase of production has been shown to improve final weights and promote health in certain situations. Objective disease detection couples remote data collection sensors with robust detection algorithms. This method has been shown be more accurate than visual observation and

detects disease prior to manifestations of obvious symptoms. Improvements in detection methods will improve animal health and welfare, as well as improve efficacy of treatment interventions and reduce the negative effects of sub-clinical morbidity

CHAPTER 2

EFFECTS OF *Mannheimia haemolytica* CHALLENGE WITH OR WITHOUT SUPPLEMENTATION OF *Saccharomyces cerevisiae boulardii* STRAIN I-1079 ON IMMUNE UPREGULATION AND BEHAVIOR IN BEEF STEERS

Introduction

Objectives of this experiment were examine the effects of live yeast (LY) supplementation on immunological, physiological and behavioral responses in steers experimentally challenged with *Mannheimia haemolytica* (MH). Thirty-six crossbred Angus steers (BW = 352 ± 23 kg) seronegative for MH were allocated within a 2 X 2 factorial arrangement: Factor 1= roughage-based diet with or without LY (*Saccharomyces cerevisiae boulardii* I-1079, 25 g/hd/d), Factor 2 = bronchoselective endoscopic inoculation with MH or phosphate buffer solution (PBS). Steers were fed their respective diets for 28 d prior to MH challenge on day 0. Reticulo-rumen temperature (RUT; ThermoBolus, Medria) was measured continuously at 5-min intervals and rectal temperature was measured on days -4, 0 - 3, 5, 7, 10, and 14. Challenge with MH increased ($P < 0.05$) RUT from 2 to 24 h following inoculation, reaching a nadir (> 41 °C) from 9 to 11 h post challenge. Rectal temperature was increased ($P < 0.04$) for MH steers the day following inoculation. Supplementation with LY increased ($P < 0.05$) rectal temperature on days 0, 7 and 10. There were inoculation x day interactions ($P < 0.01$) present for lymphocytes, neutrophils, leukocytes and haptoglobin concentration. Steers challenged with MH had increased ($P < 0.05$)

neutrophils from days 1 to 3, total leukocyte count on days 1 and 2 and haptoglobin concentration on days 1 to 5 post challenge. Steers supplemented with LY exhibited increased ($P < 0.02$) cortisol throughout the study. Following inoculation, MH-challenged steers exhibited reduced ($P < 0.05$) DMI, eating rate, frequency and duration of bunk visit (BV) events. Results from this study demonstrate that the experimental challenge model effectively stimulated acute immune responses and behavioral changes that are synonymous with naturally occurring BRD. However, supplementation with LY minimally altered the impact of the MH challenge on physiologic and behavior traits in this study. Continuously measured RUT was more sensitive at detecting febrile responses to MH challenge than rectal temperature. These results serve to guide future research on behavioral and physiologic changes that animals exhibit during a BRD infection.

There is a renewed sense of importance to the development of animal health strategies that reduce the use of antibiotics in livestock production, which has increased the emphasis placed on disease prevention and antibiotic alternatives. In order for the beef industry to reduce the use of antibiotics, suitable alternatives are a prerequisite. Bovine respiratory disease (BRD) continues to cause economic losses and accounts for 67 to 87% of the morbidity events within feedyards (Edwards, 1996). One of the challenges with treatment of BRD is that detection of the disease is difficult and current industry approaches rely on visual appraisal of clinical symptoms (Broom, 2006), which are not always accurate. Current estimates show that visual observation is highly specific (0.92) but not very sensitive (0.27), indicating that many animals contract BRD but are

not detected (Timsit et al., 2016). The inability to detect BRD is one of the primary reasons that direct-fed antibiotics are effective.

Potential non-antibiotic alternatives are direct-fed microbials (DFM), which the FDA classifies as a source of naturally occurring live microorganisms (Yoon and Stern, 1995). Yeast products such as *Saccharomyces cerevisiae* are widely used in commercial ruminant production systems, specifically dairy operations as beneficial production responses (eg. milk yield) have been demonstrated (Beauchemin et al., 2006). There is also some evidence to demonstrate that supplementation with *S. cerevisiae* is effective at reducing morbidity in stressed calves (Zinn et. al., 1999). *Saccharomyces cerevisiae boulardii* (LY) is a subspecies of *S. cerevisiae* and is one of the most widely studied microorganisms due to its application in human patients to combat enteric diarrhea (Łukaszewicz, 2012). Supplementation with DFM could potentially serve as a non-antibiotic approach to improving health of newly received or stressed cattle (Duff and Galyean, 2007).

Objectives of this experiment were examine the effects of live yeast (LY) supplementation on immunological, physiological and behavioral responses in steers experimentally challenged with *Mannheimia haemolytica* (MH).

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M

University Institutional Animal Care and Use Committee (IACUC # 2015-0379) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2015-068).

Animals

A total of 36 Angus crossbred steers (initial BW = 386 ± 25 kg) originating from the McGregor and Beef Cattle Systems herds belonging to Texas A&M University were used in this study. All animals were considered clinically healthy based upon daily observations for the 28 d prior to challenge and were seronegative for *M. haemolytica* (MH) determined by paired serum samples collected 45 d apart. Furthermore, all animals were confirmed negative for persistently infected bovine viral diarrhea virus (BVDV), through collection of an ear notch on day -45, which was analyzed with the BVD antigen-capture ELISA (BVD-Ag ELISA).

Experimental Design and Treatment Arrangements

Steers were stratified by herd origin, initial BW, MH titer dilution, exit velocity and pre-study ADG, and a random number generator used to assign steers to 1 of 4 treatments (9 hd/treatment) arranged in a 2 x 2 factorial array. Factor 1 being a roughage-based diet without (CON) or with added LY (*Saccharomyces cerevisiae boulardii* strain I-1079 at 25 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition), and Factor 2 being bronchoselective endoscopic inoculation with MH or phosphate buffer solution (PBS). Therefore, the 4 treatments groups were (n = 9): (1) MH-CON, (2) MH-LY, (3) PBS-LY, and (4) PBS-CON.

This experiment lasted 18 d, and was conducted within a larger 84 d growth and performance study. Throughout the study, all animals were housed in 4 pens equipped with electronic feedbunks at Texas A&M University's Beef Cattle Systems Research Center in College Station, TX. Steers were segregated in pens by their dietary treatment, and challenge treatments were comingled within each pen. Therefore, steers in 2 pens were fed the CON diet, and in the other 2 pens the LY diet, with equal number of PBS and MH treatment animals within pen. Steers were offered the diets *ab libitum*, which was provided twice daily at 0700 and 1600 h. The diet (DM basis) contained 36.5% dry rolled corn, 24% corn dried distillers grains, 30% chopped alfalfa hay, 5% molasses, 2.5% dry mineral and 2% premix. The premix was composed primarily of corn dry distillers grains with either LY or isocaloric isonitrogenous placebo. Targeted intake of LY in this study was 25 g per hd daily, therefore the inclusion of LY into the premix was adjusted for level of feed intake. Both the LY and CON diets were analyzed weekly to ensure that, the LY diet maintained a level of colony forming units (CFU) of LY strain I-1079 at or above the prescribed level for this study of 1×10^{10} per d. Throughout the study, no I-1079 or wild yeast colonies were detected in the CON diet. Diets were fed by hand and the feed mixer was flushed with chopped hay after mixing the LY diet and the hay used during the flush was not fed to animals in the experiment.

Inoculation Preparation and Procedure

The *M. haemolytica* inoculum was prepared as described by Mosier et al. (1995). Briefly, *M. haemolytica* serotype A1 was grown on trypticase soy agar containing 5%

sheep blood for 18 h at 37 °C in 7% CO_2. Colonies were inoculated into brain-heart infusion broth and incubated for 16 to 18 h at 37 °C with aeration. The bacteria were then centrifuged at 3,000 × g for 15 min at 4 °C and washed with PBS twice. After the second wash, the bacteria were centrifuged as before and the pellet was re-suspended in PBS at a final concentration of 1.2 to 1.4 × 10^9 CFU/10-mL dose. After preparation, the inoculum was placed on ice in a dark cooler and transported to the site of inoculation (approximately 17 km).

On day 0, all animals were inoculated with either MH or PBS. To avoid the chance of PBS animals receiving MH via contamination of the instruments used for inoculation, the PBS treatment group was inoculated prior to the MH treatment group. The inoculations were performed with an endoscope as described by Theurer et al. (2013). Steers were restrained in a standard squeeze chute that allowed more specific restraint of the head. An endoscope 1 meter in length was inserted into the ventral meatus of one nostril and passed into the trachea to the level of the right apical lung lobe bronchi allowing visualization of the opening. A sterile bronchoalveolar lavage tube was inserted into the endoscope portal and passed until the tip of the lavage tube was visible emerging from the endoscope. Thereafter, the lavage tube was advanced another 1 to 2 cm into the opening of the right apical lung lobe bronchi. Once in place, steers in the PBS treatment group were administered 10 mL of PBS followed by a 60 mL flush of PBS (total PBS = 70 mL). Following treatment of all the PBS animals, the endoscope was disinfected with chlorhexidine solution and rinsed with saline. Subsequently, steers

in the MH treatment groups were challenged with 10 mL of *M. haemolytica* serotype A1 at 1.2 to 1.4 × 10^9 CFU/mL followed by 60 mL of PBS for a total of 70 mL.

Data Collection

Temperature Monitoring and Clinical Illness Scoring. Rectal temperatures were recorded using a digital thermometer (Cooper TM99A, Cooper-Atkins Corporation, Middlefield, CT) on days -4, 0, 1, 2, 3, 5, 7, 10 and 14. In addition, radiofrequency biothermal boluses (ThermoBolus, Medria, Châteauborg, France) were inserted into the rumen of all steers prior to initiation of the study. The ThermoBolus continuously recorded reticulo-rumen temperature (RUT) at 5-min intervals. A proprietary algorithm was used to remove variation in RUT due to drinking events. Summary statistics of RUT were computed on a daily basis for the duration of the experiment and an hourly basis from 24 h pre- to 48 h post-inoculation. All steers were monitored by two experienced evaluators twice daily throughout the duration of the experiment for clinical signs of BRD. The visual evaluation employed in the experiment has been described in detail by Step et al. (2008). The criteria included signs of depression, inappetence and respiratory distress. Evaluators assigned a severity score of 1 to 4; where, 1 was assigned for mild, 2 for moderate, 3 for severe and 4 for moribund. Steers receiving a 3 or greater were pulled from the pen and given a full medical evaluation. Rectal temperature was measured during the medical evaluation and if it exceeded 40.5 °C anti-microbial therapy was administered. All steers were returned to their home pen after the evaluation. Temperature readings, BW and treatments were recorded for every animal

that was examined for clinical signs consistent with BRD. The first treatment administered to steers suffering from BRD was tulathromycin (Draxxin, Zoetis, Parsippany, NJ) at a dosage rate of 2.5 mg/kg of BW. If the initial treatment was ineffective and the steers were still suffering after 7 d, the antimicrobial administered was ceftiofur hydrochloride (Excenel, Zoetis, Parsippany, NJ) at a dosage rate of 2.2 mg/kg of BW.

Serum Haptoglobin and Cortisol. Blood samples (10 mL; Vacutainer with no additive, Becton, Dickson and Company, Franklin Lakes, NJ) were collected via jugular venipuncture with an 18-gauge needle on days -4, 0, 1, 2, 3, 5, 7, 10 and 14. After collection samples were immediately placed in a cooler on ice. Blood samples were centrifuged at 3,000 x g for 20 min at 20 °C, and duplicate serum aliquots stored at -20 °C until subsequent analysis. Haptoglobin concentration was determined at the West Texas A&M University Ruminant Health and Immunology Laboratory (Canyon, TX) with a commercial, bovine-specific sandwich ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR). The haptoglobin analysis had an interassay CV of 16.1%. Serum concentrations of cortisol were determined as described by Littlejohn et al. (2016) and Burdick et al. (2009). A solid phase radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX) using antiserum-coated tubes were prepared according to the manufacturer's directions. Serum cortisol concentrations were determined by the average of duplicate unknown samples compared with a standard curve generated from known concentrations of cortisol using Assay Zap software

(Biosoft, Cambridge, UK). The minimum detectable cortisol concentration for this assay was 1.2 ng/mL, and the interassay CV was 8.8 %.

Hemogram. Blood samples (7 mL; EDTA, Becton, Dickson and Company, Franklin Lakes, NJ) were collected via jugular venipuncture with an 18-gauge needle on days -4, 0, 1, 2, 3, 5, 7, 10 and 14, relative to challenge. Samples were immediately submitted to a commercial lab (Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX) for total and differential leukocyte determination, total erythrocytes, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets. Blood counts were performed with an automated hemocytometer (ADVIA 120, Siemens Healthcare Diagnostics, Tarrytown, NY) using the factory installed cattle setting (ADVIA 120 Multispecies System Software, Version 2.206 MS, Siemens Healthcare Diagnostics). The hemocytometer counts leukocytes, erythrocytes and platelets by optical scatter and fluorescence. Hemoglobin concentration is determined by the cyano-methemoglobin technique. Differential leukocyte percentages were determined by counting cells on modified blood smears and absolute counts were calculated using the total leukocyte count from the hemocytometer.

DMI and Feeding Behavior. Pens were equipped with electronic feedbunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake and feeding behavior data on an individual-animal basis. The GrowSafe system consisted of feed

bunks equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of EID tags. Assigned feed disappearance (AFD) rates were computed daily for each feed bunk to assess data quality and averaged 98% throughout the 84 d study. Feeding behavior traits were based on frequency and duration of bunk visit (BV) events. A BV event commenced when the EID of an animal was first detected, and ended when the time between consecutive EID recordings exceeded 100 s, was detected at another feed bunk, or when the EID of another animal was detected at the same feed bunk (Mendes et al., 2011). Bunk visit frequency was defined as the number of independent events recorded regardless of whether or not feed was consumed, and BV duration as the sum of the lengths of all BV events recorded during a 24-h period. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance during each BV event. A subroutine of the GrowSafe 6000E software (Process Feed Intakes) was used to compute daily feed intake. For this study, eating rate was computed as the ratio of daily DMI to daily BV duration.

Statistical Analysis

This experiment was designed as a randomized complete block with a 2 x 2 factorial treatment arrangement with animal serving as the experimental unit. Temperature measures, feeding behaviors, haptoglobin, cortisol and hemogram measurements were analyzed using the MIXED procedure (SAS 9.4, SAS Institute Inc., Cary, NC) with an autoregressive covariance structure. The model for all variables

included the main effects of diet, inoculation, time (d or hour) and all possible interactions. Initial exit velocity, which is a measurement of animal temperament was used as a covariate for all analyses. Although steers were randomized into treatments with pre-study exit velocity as a consideration, there were differences between treatments on days -28 and -14 of the study. Therefore, initial exit velocity was used as co-variate to remove any effect of temperament on dependent variables. When a main effect x time interaction ($P \leq 0.05$) was detected the SLICE output option was used to determine mean separation of main effects for each time point. In the event a diet x inoculation or a diet x inoculation x day interaction was detected ($P \leq 0.05$), least squares means were separated using the PDIFF multiple comparison test. Growth rates for individual steers were modeled by linear regression of body weight measurements on study day using PROC GLM (SAS, 9.4). The regression coefficients were used to compute ADG, initial body weight and final body weight.

Results

There were no diet x inoculation interactions detected for initial BW, ADG, or final BW, and there were no main effect differences between the inoculation or dietary treatments for initial BW. Steers inoculated with MH had reduced ($P < 0.03$) ADG (0.67 vs. 1.21, kg/d) compared to the PBS-challenged steers and there was a tendency ($P < 0.08$) for LY supplementation to improve ADG (1.15 vs. 0.74, kg/d) relative to the CON steers. Although, caution should be taken when interpreting the results, because ADG was only measured over 18 d. Final BW was not impacted by diet or inoculation

treatments, however final BW of MH-challenged steers was numerically lower (P = 0.17) by 14 kg compared to PBS-challenged steers.

Clinical illness scores were not impacted by the inoculation treatment (P = 0.65). The evaluators were not blinded to the treatment assignments, but the animals were comingled within pen and there was no way to visually identify which inoculation the steers received when making the evaluation. Throughout the study, only 1 steer (MH treatment) received a clinical illness score ≥ 3 and during the health evaluation exhibited a rectal temperature > 40.5°C. There were no re-treatments or mortalities throughout the study. There was a day effect (P < 0.01) detected for clinical illness scores. On days 4 and 5 post-inoculation, animals had the greatest clinical illness scores (0.63) which were different (P < 0.05) from the remaining 8 d post-inoculation (average = 0.10). Following inoculation, there was a heat wave, during which the ambient high temperature averaged 35°C and relative humidity averaged 96%. These environmental factors likely impacted the accuracy of the clinical illness scoring system, and heat stress may have influenced the clinical illness scores > 0, that were recorded for PBS animals as heat stress symptoms share commonality with mild BRD symptoms. At harvest the lungs of all study animals were inspected for lesions or abnormalities. The steer in the MH treatment that was treated had a small consolidation in the cranioventral region of the right lung lobe, the lungs of the remaining steers were grossly normal.

Steers challenged with MH exhibited rapid increases in RUT almost immediately following inoculation (Fig. 1), from 2 to 24 h post MH challenge, reaching a nadir (> 41°C) from 9 to 11 h post MH challenge. There were no differences between the MH

and PBS inoculation treatments 18 h post MH challenge. There were no main effect differences in rectal temperature between the inoculation treatments, however, an inoculation x day interaction ($P < 0.01$; Table 1) was detected. Steers challenged with MH had greater rectal temperature on days -4 and 1, compared to PBS-challenged steers. Overall, steers supplemented with LY exhibited greater ($P < 0.02$) rectal temperature (39.64 vs. 39.46, °C) opposed to the CON steers. Furthermore, there was a dietary treatment x day interaction ($P < 0.02$), with LY having greater rectal temperature on days 0, 7 and 10 compared to CON steers. Challenge with MH increased overall RUT ($P < 0.03$) and there was a tendency ($P < 0.07$) for an inoculation x day interaction. Steers challenged with MH had greater ($P < 0.01$) RUT on day 0 and a tendency ($P < 0.09$) for increased RUT on days 1 and 2. There were no differences in RUT due to dietary treatment and no inoculation x diet interaction detected.

Total leukocyte, differential leukocyte count, haptoglobin and cortisol data are shown in Table 2. There was an inoculation x diet x day interaction ($P < 0.01$) for MCHC (Fig. 2 and Table 3). This resulted in decreased ($P < 0.05$) MCHC for the MH-LY treatment on days 1, 2 and 3 compared to the MH-CON treatment with the PBS-CON and PBS-LY treatments being intermediate. Furthermore, on day 5 the PBS-CON treatment had increased ($P < 0.05$) MCHC relative to the MH-CON and the MH-LY and PBS-LY treatments were intermediate. On day 10 both PBS-CON and PBS-LY were greater ($P <0.05$) than MH-CON and the MH-LY treatment was intermediate. There was a diet x day interaction ($P < 0.04$) detected for concentration of basophils, which resulted in increased concentration on day 2 for the CON compared to the LY steers. Graphical

analysis of the basophils (not presented) revealed multiple slope changes for both dietary treatments, which is most likely the reason for the significant interaction term. The separation between the treatments was also not biologically relevant. An inoculation x day interaction ($P < 0.01$) was detected for concentration of lymphocytes (Fig. 3, Panel A), although the subclass means were not different ($P > 0.05$) from each other for any given day. However, MH challenge stimulated overall neutrophil production ($P < 0.01$; Fig. 3, Panel B) and the concentration was dependent upon day ($P < 0.01$). Neutrophils increased rapidly following MH inoculation reaching a nadir on day 1 and maintained separation from PBS-challenged steers on days 2, 3, 7 and 10. In addition, MH challenge stimulated an increase ($P < 0.02$) in overall leukocyte production (Fig. 3, Panel C), which also interacted with day ($P < 0.01$). Total leukocytes increased following challenge peaking on day 1 and was greater than PBS-challenged steers on day 2, after which levels returned to normal. Platelets responded with an inoculation x day interaction ($P < 0.01$; data not shown), where they were greater ($P < 0.05$) for PBS-challenged steers on day 5, but not different for any other day. Mean seperation between the inoculation treatments were minimal and of the same pattern up to and after day 5, although on day 5 the PBS-challenged steers exhibited a spike in platelets. The cause and relevance of this spike is unknown.

Haptoglobin concentrations exhibited the strongest response of all serology measures to MH challenge. Steers challenged with MH on average exhibited a 15-fold increase ($P < 0.05$; 33.6 vs. 2.1 ng/ml) in haptoglobin concentration compared to the PBS steers. There was also an inoculation x day interaction ($P < 0.05$; Fig. 3, Panel D)

detected for haptoglobin concentration, MH-challenged steers had greater haptoglobin on days 1, 2, 3 and 5 compared to PBS-challenged steers. The haptoglobin concentration in MH steers increased rapidly reaching a nadir on day 3 and returning pre-inoculation levels on day 10. There were no interactions detected between dietary and inoculation treatments for cortisol, or differences between inoculations. However, steers supplemented with LY had greater ($P < 0.02$) circulating cortisol (34.4 vs. 26.1, ng/mL) than the CON fed steers throughout the study.

There were inoculation x day interactions ($P < 0.01$) detected for DMI, BV frequency, BV duration and eating rate (Fig. 4). Steers inoculated with MH displayed a sharp decline in DMI, which was less ($P < 0.05$) compared to the PBS-inoculated steers days 0 to 3 post inoculation. Frequency of BV events was reduced ($P < 0.05$) the day of inoculation for MH-challenged steers compared to the PBS-challenged steers. Similarly, BV duration was less ($P < 0.05$) on day 0 for MH-challenged steers although, it was greater ($P < 0.05$) on day 11 compared to PBS-challenged steers. The impact on eating rate due to MH challenged was more delayed than the other feeding behaviors. The MH-challenged steers had reduced ($P < 0.05$) eating rate on days 5, 7 to 12 and 14 compared to the PBS-challenged steers.

Discussion

The results of this study show that the challenge model successfully initiated immunological signs of disease, however, the model did not induce clinical signs of disease. Gross clinical signs of disease resulting from the challenge model were not

expected. Previous studies using a similar MH strain with intra-tracheal delivery, reported that challenged animals appeared clinically normal or slight increases in clinical illness scores that were not different from clinically normal (Capik et al., 2015; Corrigan et al., 2007).

Body temperature is a key measurement used in livestock production to inform decision paradigms on animal health status. Temperature is the most common and often the only objective measurement in determination of BRD. However, animals in a febrile state may not display clinical signs of disease. Capik et al. (2015) examined the transmission dynamics among beef calves experimentally challenged with MH and reported a weak association between clinical illness signs and rectal temperature. Timsit et al. (2011) monitored RUT in young bulls after arrival to a feedyard to assess its value for preclinical detection of BRD. Reticulo-rumen hyperthermia exhibited a positive predictive value of 73%, and in the bulls that were correctly identified the hyperthermia alert was detected 1 to 3 d prior to observed clinical symptoms. In the current study, inoculation with MH created a rapid increase in RUT that returned to normal within 24 h of inoculation. Rose-Dye et al. (2011) reported nearly identical RUT patterns in steers that were intra-trachealy challenged with MH. Although, RUT was only statistically greater for the 24 h post-inoculation, there was a tendency for increased RUT on days 2 and 3 following inoculation. These differences and tendencies accumulated to create an overall main effect increase in RUT for the MH inoculation treatment. Results from RUT are easier to interpret than the rectal temperature and are in agreement with the other biological markers of inflammation. Steers challenged with MH exhibited an

increase in rectal temperature the day after challenge. Previous reports have shown similar transient increases in rectal temperature due to MH challenge. Corrigan et al. (2007) and Hanzlicek et al. (2010) reported increases in rectal temperature the day of challenge, although animals returned to pre-challenge levels within 24 h. Theurer et al. (2013) and Burciaga-Robles et al. (2010) both reported increases in rectal temperature that initiated < 6 h after inoculation and persisted for 24 h. The current study measured rectal temperature once per day, while the other aforementioned studies measured rectal temperature on varying serial hour schedules throughout the initial 24 h. Measuring rectal temperature only once per day may have introduced more variance into the measurement resulting in the differences observed between the dietary treatments and the challenge treatments prior to challenge administration. Hanzlicek et al. (2010) measured rectal temperature 3 times per day on beef steers experimentally challenged with MH. They reported that the 3 measurements were statistically different from each other and was greatest (morning = 39.6, noon = 39.4 and evening = 40.2 °C) in the early evening. It seems biologically incorrect that supplementation with LY would increase body temperature and there is no evidence in the literature to support these findings. In fact, Zhu et al. (2016) reported that there was a linear tendency for reductions in rectal temperature with increasing supplementation levels of *S. cerevisiae* in dairy cattle under heat stress. Also, in the current study there were no increases in RUT for steers supplemented with LY. In fact, there were no differences in RUT between the dietary treatments at any time point. In the current study these differences are believed to be environmental interference or error. Hanzlicek et al. (2010) commented that typical

clinical measurements for detecting disease such as rectal temperature, heart rate and respiratory rate yielded conflicting results and varied throughout the day in animals challenged with MH. Furthermore, rectal temperature measurements in their study exceeded their upper reference (39.5 °C) limit throughout the study, which was attributed to high environmental temperatures and the restraint stress associated with the physical examination. Results from this study and others suggest that RUT may be a more accurate measurement for identifying febrile animals.

Mean corpuscular hemoglobin concentration is the cellular hemoglobin concentration per average erythrocyte and is calculated as the hemoglobin to hematocrit ratio (Smith et. al., 2015). The most common cause of a slightly decreased MCHC is a regenerative anemia (Jones and Allison, 2007). Hanzlicek et al. (2010), reported a reduction in MCHC in crossbred beef steers on day 3 following a MH challenge. Burciaga-Robles et al. (2010) did not report MCHC, however, they found reductions in MCH, (ratio of hemoglobin to erythrocytes) which is similarly reduced by a regenerative anemia (Smith et. al., 2015). Contrary to these studies, Corrigan et al. (2007) observed no change in MCHC in response to MH challenge. In the current study there was a tendency for an inoculation x day interaction for MCHC (not presented), graphically MCHC was reduced on days 5 and 10. The pattern resembled the minimal transient differences reported by Hanzlicek et al. (2010), although there appears to be a greater day effect in the current study. There are disagreements on the normal range of MCHC within the literature; Smith et al. (2015) 36–39 g/dL, Jones and Allison (2007) 32–36 g/dL and Kansas State University Clinical Pathology Laboratory 33–37 g/dL. However,

published ranges are relatively similar and MCHC values observed in the current study are generally within those limits. During the 3 d following MH inoculation, the MH-CON steers exhibited greater ($P < 0.05$) MCHC relative to the MH-LY steers, with PBS-CON and PBS-LY steers being intermediate. While these results are not consistent with findings from the aforementioned studies, there were marked reduction in MCHC for all treatments on days 5 and 10 post MH challenge. The MH-CON treatment exhibited less ($P < 0.05$) MCHC than PBS-CON on d 5, and PBS-CON and PBS-LY on day 10. Although, these differences are statistically different, the magnitude of mean separation (-4 and -3%, respectively) is small and the biological relevance of this difference remains to be determined. *M. haemolytica* possess multiple virulence factors that contribute to pathogenesis. Primarily, a lipopolysaccharide complex that causes hemorrhage, edema, acute inflammation and a leukotoxin that leads to lysis of leukocytes and platelets (Griffin et. al., 2010). However, the increase in MCHC in the MH-LY steers may be due to slight reductions in hemorrhage induced by the innate immune response at the inoculation site.

Steers challenged with MH exhibited altered leukograms synonymous with a bacterial infection (Jones and Allison, 2007). Although, there were no differences between inoculation treatments on any given day, lymphocytes for MH challenge steers were numerically lower 24 h post-inoculation. Burciaga-Robles et al. (2010) and Gånheim et al. (2003) both reported decreases in lymphocytes resulting from intra-tracheal inoculation with MH. In contrast, Corrigan et al. (2007) and Hanzlicek et al. (2010) reported no differences in lymphocytes associated with MH challenge.

Lymphopenia is associated with stress, endotoxemia, and acute viral and bacterial infections (Jones and Allison, 2007). There were considerable differences in lymphocyte count across the studies and where differences were observed, there were overall greater lymphocytes recorded. Therefore, the conflicting results across studies may be due to a host-bacterial interaction or dependent upon the total number of lymphocytes prior to challenge.

Neutrophils are the predominant leukocyte in mammals and can migrate from storage in bone marrow into circulation within 2 h of insult (Smith, 2015; Tizard, 2013). Neutrophilia occurs in response to infectious processes, tissue injury, neoplastic diseases and non-inflammatory conditions (Jones and Allison, 2007). Steers challenged with MH exhibited nearly a 3-fold increase in neutrophils within 24 h of inoculation. Neutrophilia in response to MH challenge is commonly reported (Burciaga-Robles et al., 2010; Corrigan et al., 2007; Gånheim et. al., 2005 and Hanzlicek et. al., 2010) and a reliable indicator that the challenge model successfully produced an acute-immune response.

Total leukocyte count results in the present study are consistent with previous reports (Burciaga-Robles et al., 2010; Corrigan et al., 2007; Gånheim et. al., 2005 and Hanzlicek et. al., 2010) in which total leukocytes were elevated for 24 to 48 h after MH challenge. Naturally the increase in leukocyte count is in part due to the increase in neutrophils. Haptoglobin is an acute phase protein that performs multiple roles during the upregulation of the innate immune response. Through the binding of free hemoglobin in the blood, haptoglobin stabilizes iron reducing oxidative stress (Ceciliani et al., 2012) and imposes a bacteriostatic effect by making iron unavailable for proliferating bacteria

(Idoate et al., 2014). The haptoglobin-hemoglobin complex also exerts anti-inflammatory effects, when this complex binds with macrophages they in turn release anti-inflammatory cytokines such as interlukin-10 (Ceciliani et al., 2012). Haptoglobin has been proven an effective measure of diagnosis and prognosis for multiple disease processes in beef cattle including BRD (Eckersall and Bell, 2010; Godson et al., 1996). In the current study, steers challenged with MH exhibited a marked increase in haptoglobin concentration 24 h after MH challenge which persisted for 5 d. In contrast, PBS challenge steers exhibited a minimal haptoglobin response during the study. Similarly, Burciaga-Robles et al. (2010) reported increased concentrations of haptoglobin for 4 d following MH challenge. Although Corrigan et al. (2007), did not reported statistical differences between days, haptoglobin concentration of MH challenged animals were 4-fold greater 5 d after challenge than the baseline measurements. These results further indicate that the challenge model adequately stimulated an immune response in this study.

Supplementation with LY did not impact the leukogram or haptoglobin concentrations. However, steers supplemented with LY exhibited increased circulating cortisol levels. The impact of yeast DFM supplementation on the hypothalamic-pituitary-adrenal axis is unclear. In agreement with the current study, Buntyn et al. (2014) reported increased circulating cortisol in steers supplemented with live *S. cerevisiae* prior to challenge, however, post endotoxin challenge the levels were below control animals. They also reported a reduction in pro-inflammatory cytokines (tumor necrosis factor-alpha, interlukin-6 and interferon-gamma) during the period, in which

cortisol was elevated. Buntyn et al., (2014) concluded that yeast supplementation may reduce negative effects associated with the innate immune inflammatory response through this cortisol pro-inflammatory cytokine messaging mechanism. Increased levels of cortisol due to supplementation with yeast DFM have also been reported in other species. Iwashita et al. (2014) reported increases of cortisol in juvenile tilapia following 4 wk of supplementation with *Bacillus subtilis*, *Aspergillus oryzae* and *S. cerevisiae*. The elevated cortisol levels were only observed on the initial measurement and were not different from the controls during the 2-wk period following disease challenge. However, supplementation with DFM improved feed conversion (24%, $P < 0.05$) and increased the survivability rate to an intra-peritoneal challenge with *Aeromonas hydrophilia* (21.3 vs 5.3%, $P < 0.05$) and *Streptococcus iniae* (34.7 vs 4.3%, $P < 0.05$). It is not possible to determine if the positive effects observed were collective influence of the 3 DFM's fed in combination or if it was due to S. cerevisiae. Contrary to these findings, Zaworski et al. (2014) reported decreased concentrations of cortisol in postpartum dairy heifers supplemented with *S. cerevisiae* (fermentation product) compared to the controls. Burdick-Sanchez et al. (2013) reported no differences in heifers supplemented with *S. cerevisiae* (cell wall) prior to an intravenous challenge with endotoxin. However, post-challenge heifers supplemented with *S. cerevisiae* exhibited reduced concentrations of cortisol as well as interlukin-6. Similarly, Collier et al. (2011) reported supplementation with *S. cerevisiae boulardii*, reduced cortisol, interferon gamma and interlukin-6 post intra-venous endotoxin challenge in newly weaned piglets. Reduction in interlukin-6 concentrations due to yeast supplementation appears to be

fairly repeatable; however, the impact that yeast supplementation exerts upon the relationship between cortisol and pro-inflammatory cytokines is yet to be determined. Circulating cortisol patterns in the current study were not impacted by the challenge model as seen in the aforementioned studies. Even though pro-inflammatory cytokines were not measured in this study, there appears to be no impact on other measures of inflammation (body temperature, haptoglobin, neutrophils) impacted by cortisol levels or supplementation with LY.

When animals become morbid they enter into a state of hyper-metabolism, due to increased energy demands required to produce pro-inflammatory cytokines, acute phase proteins, lymphocytes and mount a febrile response (Carroll and Forsberg, 2007). In human patients, Long (1977) observed alterations of resting metabolism measured by indirect respiratory calorimetry, and reported a maximum increase in resting metabolism of 45% when the patient had peritonitis. To compensate for these increased energy demands, mammals display various behavioral responses such as increased sleep, and reductions in social activity, sexual behavior and feed intake in an effort to conserve energy (Carroll and Forsberg, 2007). Lethargy and reduced feed intake have been well documented in studies monitoring the health of beef cattle (Sowell et al., 1999; Quimby et al., 2001; Jackson et al., 2016; Hutcheson and Cole, 1986 and Galyean and Hubbert, 1995). These metrics are often the basis for subjective disease detection paradigms. In the current study, steers challenged with MH had reduced DMI, reductions in frequency and duration of BV events as well as reduction in eating rate. The reduction in frequency and duration of BV events only occurred the day following MH challenge, whereas,

DMI remained suppressed for 4 d following MH challenge. Theurer et al. (2013), reported decreases in duration of time calves spent at a hay feeder that were similarly inoculated with MH. Interestingly, in the same study the duration the MH calves spent at a grain feeder was less only 24 h after inoculation and returned to normal, as though the calves were self-selecting a higher energy diet to meet increased requirements. These alterations in behavior, which are synonymous with behavior changes observed in naturally occurring BRD cases, further illustrate the effectiveness of the challenge model.

Although the intent of this study is not to evaluate the current methodologies for determining BRD in cattle, it is worth highlighting that in many of the challenge studies discussed, very few reported gross clinical signs of disease typically observed in a commercial setting. However, they all provide evidence of physiologic changes that are suggestive of a morbid/inflammatory state. There have been recent efforts to evaluate the effectiveness of using visual observation for disease detection. Comparing the presence of lung lesions at slaughter with previous animal treatment records White and Renter (2009) reported that visual observation was 62% sensitive and 63% specific. Timsit et al. (2016) using the studies included in the White and Renter (2009) analysis and others, concluded that the sensitivity of visual observation was only 27% sensitive, however the specificity was 92%. The differences between the studies were impacted by the different estimates used in the Bayesian estimation, with Timsit et al. (2016) results more closely resembling the averages of the studies. Regardless, these studies illustrate the challenge with relying on visual observation to detect BRD, and demonstrate that BRD cases are

often not identified. There are multiple reports that highlight behavioral changes prior to clinical signs of disease. Pillen et al. (2016) reported reductions in motion index and steps per day, 5 and 4 d prior to diagnosis, respectively. Jackson et al. (2016) used a 2-slope broken-line regression model to characterize deviations in DMI and feeding behavior patterns preceding the onset of observed clinical symptoms associated with BRD. The model-detected breakpoint for DMI occurred 6.8 d prior to observed clinical illness, whereas, breakpoints for BV frequency and duration were 7.6 and 7.2 d prior to observed clinical illness. Quimby et al. (2001), monitored BV traits with a cumulative summation chart and was able to detect morbidity on average 4.1 d prior to clinical symptoms with an overall accuracy and sensitivity of 87 and 90 % respectively. White et al. (2015), developed a system that monitors a suite of behavior traits that was capable of detecting morbid animals 0.75 d prior to visual observation. These results highlight that remotely obtained continuously recorded behavior variables are able to detect behavior changes prior to visual observation. Combined results from these reports illustrate there is opportunity to improve disease detection methods in the beef industry.

Conclusion

In this study we evaluated the impacts of an experimental *M. haemolytica* challenge model on physiologic and behavior parameters of crossbred beef steers. The model effectively stimulated inflammation, upregulated leukocytes and acute phase proteins, synonymous with an acute immune response associated with naturally occurring BRD. Furthermore, this challenge model impacted feeding behavior similarly

to an acute BRD infection. Supplementation with LY minimally impacted performance, physiologic or behavior responses in this study. Future research endeavors should seek out the relationship between LY supplementation and cortisol. This study highlights that physiologic and behavior changes associated with BRD are difficult to detect through visual observation and may guide future research on the behavior changes associated with BRD.

CHAPTER 3

EFFECTS OF *Mannheimia haemolytica* CHALLENGE WITH OR WITHOUT SUPPLEMENTATION OF *Saccharomyces cerevisiae boulardii* STRAIN I-1079 ON ANIMAL PERFORMANCE AND BEHAVIOR IN BEEF STEERS

Introduction

Objectives of this experiment were to examine the effects of live yeast (LY) supplementation on growth, feed efficiency, physiological and behavioral responses in growing beef steers prior to and following experimental challenge with *Mannheimia haemolytica* (MH). Thirty-six crossbred steers (BW = 352 ± 23 kg) seronegative for MH were allocated within a 2 X 2 factorial treatment arrangement: Factor 1 = roughage-based diet with or without LY (*Saccharomyces cerevisiae boulardii* I-1079 at $1x10^{10}$ CFU/d, Lallemand Animal Nutrition), Factor 2 = bronchoselective endoscopic inoculation with MH or phosphate buffer solution (PBS). Electronic feed bunks (GrowSafe) were used to measure DMI and feeding behavior traits, and thermo-boluses (Medria) recorded reticulo-rumen temperature (RUT) at 5-min intervals. Steers were fed their respective diets for 28 d prior to challenge administration. Supplementation with LY reduced ($P < 0.05$) ADG (1.52 vs. 1.74, kg), G:F (0.14 vs. 0.16) and DMI expressed as a percentage of body weight (2.85 vs. 3.01%) during the 28 d prior to challenge. Supplementation with LY also impacted feeding behavior, with reductions ($P < 0.05$) in bunk visit (BV) frequency and time to bunk (TTB). The effects of the MH challenge were mild with no differences in clinical illness scores between the treatments. However,

steers challenged with MH had reduced ($P < 0.03$) eating rate and increased ($P < 0.02$) maximal RUT for 28 d following inoculation. The MH challenge also tended to reduce ($P < 0.06$) DMI and DMI expressed as a percentage of body weight. These differences between the MH and PBS treatments persisted throughout the duration (d 28 – 84) of the study. During the 28 d post-inoculation, steers supplemented with LY exhibited improved ($P < 0.02$) ADG (1.50 vs. 1.13, kg) and G:F (0.15 vs. 0.11). These performance improvements continued throughout the duration of the study, with LY supplemented steers having increased ($P < 0.05$) ADG (1.56 vs. 1.33, kg) and G:F (0.15 vs. 0.13). It is unclear why LY supplementation prior to challenge reduced growth, intake and efficiency. The MH challenge model induced behavior and physiologic changes in growing beef steers without creating gross signs of clinical disease. Supplementation with LY improved ADG and G:F post experimental challenge and these improvements continued throughout the duration of the study. These results suggest that LY supplementation improves growth and efficiency when the animal is stressed or the immune system is compromised.

In response to combat antibiotic resistant bacteria the US Food and Drug Administration (FDA) has taken steps to fundamentally change how antibiotics can legally be applied to feed for the production of food animals. The Veterinary Feed Directive Final Rule that was implemented in December of 2016, requires a veterinary prescription for many antibiotics previously available over-the-counter. Furthermore, the FDA is particularly interested in alternative methods to open-ended supplementation of antibiotics. Based upon judicious use principles, this will require more targeted use of

antibiotics, non-antibiotic therapeutic options or changes in management/production practices. Feed-grade antibiotics, most commonly tetracyclines, are routinely used in beef production during the feedlot phase of production to control/treat bovine respiratory disease (BRD; Agga et al., 2016). In fact, recent surveys revealed that 57% of nutritionists (Samuelson et al., 2016) and 74% of veterinarians (Terrell et al., 2011) recommended supplementing receiving diets with tetracyclines. The FDA 2015 summary report of antimicrobials sold for livestock use illustrated that the majority (73.6%) of medically important antibiotics were delivered within feed while only 3.6% were administered by injection. If the beef industry is to reduce or remove antibiotics, determination of suitable alternatives is a pre-requisite. A non-antibiotic alternative is direct-fed microbial (DFM) supplementation, which has shown to be effective at reducing morbidity in shipping stressed calves (Zinn et. al., 1999). The FDA classifies DFM as a source of naturally occurring live microorganisms (Yoon and Stern, 1995). Yeast products such as *Saccharomyces cerevisiae* are widely used in commercial ruminant production, specifically dairy production systems because improved milk yield has been well documented (Beauchemin et al., 2006). *Saccharomyces cerevisiae boulardii* (LY) is a subspecies and is one of the most widely studied microorganisms due to its application in human patients to combat enteric diarrhea (Łukaszewicz, 2012). We hypothesize that supplementation with LY will reduce the severity of morbidity, as well as improve growth and feed efficiency of beef cattle. There have been few studies measuring the effects of LY on growing beef cattle; therefore, our objectives were to quantify differences in growth, feed efficiency, ruminal temperature (RUT) and feeding

behavior between steers supplemented with LY and a negative control (CON). Furthermore, identify the effects of LY on morbidity and recovery associated with an experimental challenge of *Mannheimia haemolytica* (MH).

Materials and Methods

Animals and Experimental Design

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC # 2015-0379) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2015-068). Thirty-six Angus crossbred steers (initial BW = 353 ± 23 kg) originating from the McGregor and Beef Cattle Systems herds belonging to Texas A&M University were used in this study. All animals were seronegative for MH determined by paired serum samples collected 45 d apart on days -45 and 0. Furthermore, all animals were confirmed negative for persistently infected bovine viral diarrhea virus (BVDV), through collection of an ear notch on day-45, which was analyzed with the BVD antigen-capture ELISA (BVD-Ag ELISA). Prior to commencement of the study, steers were allocated into a 2 x 2 factorial treatment arrangement. Factor 1 being a roughage-based diet without (CON) or with added LY (*Saccharomyces cerevisiae* boulardii strain I-1079 at 25 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition), and Factor 2 being bronchoselective endoscopic inoculation with MH or phosphate buffer solution (PBS). A random number generator was used to assign steers to treatment arrangements and

individuals were stratified by source, initial BW, MH titer dilution, exit velocity and pre-study ADG. Therefore, the 4 treatments groups were (n = 9): (1) MH-CON, (2) MH-LY, (3) PBS-CON, and (4) PBS-LY.

Throughout the study, all animals were housed in 4 pens equipped with electronic feedbunks at Texas A&M University's Beef Cattle Systems Research Center in College Station, TX. Steers were segregated in pens by their dietary treatment, and challenge treatments were comingled within each pen. Therefore, steers in 2 pens were fed the CON diet, and in the other 2 pens the LY diet, with equal number of PBS and MH treatment animals within pen. Steers were offered the diets *ab libitum*, which was provided twice daily at 0700 and 1600 h. The diet (DM basis) contained 36.5% dry rolled corn, 24% corn dried distillers grains, 30% chopped alfalfa hay, 5% molasses, 2.5% dry mineral and 2% premix. The premix was composed primarily of corn dry distillers grains with either LY or isocaloric isonitrogenous placebo. Targeted intake of LY in this study was 25 g per hd daily, therefore the inclusion of LY into the premix was adjusted for level of feed intake. Both the LY and CON diets were analyzed weekly to ensure that, the LY diet maintained a level of colony forming units (CFU) of LY strain I-1079 at or above the prescribed level for this study of 1×10^{10} per d. Throughout the study, no I-1079 or wild yeast colonies were detected in the CON diet. Diets were fed by hand and the feed mixer was flushed with chopped hay after mixing the LY diet and the hay used during the flush was not fed to animals in the experiment. Steers were fed their respective treatment diets for the first 56 d of the 84 d study after which both treatments were fed the CON diet.

Throughout the study weights were collected a minimum of every 7 d and with greater frequency the 10 d following inoculation challenge. On day 28 all animals were endoscopically inoculated with either MH or PBS. To reduce to potential of cross contamination with MH all of the PBS treatment steers were inoculated prior to the MH treatment group. Endoscopic inoculations were conducted using bronchoselective endoscopy. This procedure allows selective placement of the endoscope and media into the right apical lung lobes via the tracheal bronchus (Theurer, et al., 2013). Upon insertion of the catheter into the right-side of the tracheal bifurcation 10 mL of MH serotype A1 at a dosage rate of 1.2 to 1.4 × 10^9 CFU/10-mL was administered, followed by a 60 mL flush of PBS. To mimic the procedure for the control treatment, steers were endoscopically administered with 10 mL of PBS followed by 60 mL of PBS.

Mannheimia haemolytica Preparation

The MH inoculum was prepared as described by Mosier et al. (1995). Briefly, MH was grown on trypticase soy agar containing 5% sheep blood for 18 h at 37 °C in 7% CO_2. Colonies were inoculated into brain-heart infusion broth and incubated for 16-18 h at 37 °C with aeration. The bacteria were then centrifuged at 3,000 × g for 15 min at 4 °C and washed with PBS twice. After the second wash, the bacteria were centrifuged as before and the pellet was re-suspended in PBS at a final concentration of 1.2 to 1.4 × 10^9 CFU/10-mL dose. After preparation, the inoculum was placed on ice in a dark cooler and transported to the site of inoculation.

The GrowSafe System

Pens were equipped with electronic feedbunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake and feeding behavior data on an individual-animal basis. The GrowSafe system consisted of feed bunks equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of EID tags. Assigned feed disappearance (AFD) rates were computed daily for each feed bunk to assess data quality and averaged 98% throughout the 84 d study. Feeding behavior traits were based on frequency and duration of bunk visit (BV) events, duration of non-feeding intervals (NFI), head-down (HD) duration and time to bunk (TTB). A BV event commenced when the EID of an animal was first detected, and ended when the time between consecutive EID recordings exceeded 100 s, was detected at another feed bunk, or when the EID of another animal was detected at the same feed bunk (Mendes et al., 2011). Bunk visit frequency was defined as the number of independent events recorded regardless of whether or not feed was consumed, and BV duration as the sum of the lengths of all BV events recorded during a 24-h period. The interval lengths between BV events were defined as NFI. Maximal NFI was defined as the longest NFI, and NFI SD as the SD of all NFI within each day. HD duration was computed as the sum of the number of times the EID for an animal was detected each day multiplied by the scan rate of the GrowSafe system (1.0 s). Daily TTB was computed as the interval length between time of feed-truck delivery within pen and each animal's first BV event following feed delivery. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance

during each BV event. A subroutine of the GrowSafe 6000E software (Process Feed Intakes) was used to compute daily feed intake. For this study, eating rate (ER) was computed as the ratio of daily DMI to daily BV duration. To compute meal data, a 2-pool Gaussian-Weibull distribution model was fit to log-transformed non-feeding interval data, and the intercept of the 2 distributions used to define meal criterion (Bailey et al., 2012; Yeates et al., 2001). Meal criterion was used to compute individual animal meal data (meal frequency and duration, meal length and intake). Meal duration was defined as the sum of the lengths of meal events recorded each day. Meal length (min) was calculated as the average length of a meal event per day and similarly meal intake was the average DMI per meal (kg/meal).

The Medria System

The Feed Phone system (Medria, Châteauborg, France) is composed of the ThermoBolus, Axel collar and Radio Base station. The ThermoBolus continuously recorded reticulo-rumen temperature (RUT) at 5-min intervals. A proprietary algorithm was used to remove variation in RUT due to drinking events. Summary statistics of RUT were computed on a daily basis for the duration of the experiment. Feeding and activity behaviors are generated from data recorded by the Axel sensor that is placed on a collar and securely fitted around the steers' neck. The sensor consists of a micro-electromechanical tri-axial accelerometer that quantifies changes of inclination, lateral and vertical accelerations on a continuous basis. Nine metrics are recorded over 5-min intervals, and is automatically transmitted to the radio base station and thereafter to a

web-based data center. Processing algorithms on servers convert the raw data into animal behaviors and the most dominant behavior within a 5-min interval is reported (Delagarde and Lemonnier, 2015). There are 5 reported behaviors that are mutually exclusive; ingestion, rumination, rest, other activity and over activity as well as standing which is not mutually exclusive with the other behaviors. All animals were fitted with an Axel collar and had a ThermoBolus inserted in to their rumen with the assistance of a bolus gun specifically designed for the bolus prior to the beginning of the study.

Statistical Analysis

Growth rates for individual steers were modeled by linear regression of body weight measurements on study day using PROC GLM (SAS, 9.4). The regression coefficients were used to compute ADG, initial body weight and final body weight. Similarly, estimates for missing feed intake due to system failure were derived from linear regression of feed intake on study day using PROC GLM. Daily fluctuations in DMI (Daily DMI Var) were calculated as the average difference of the observed DMI minus the predicted DMI based upon the linear regression of DMI on day of study. All performance, feed efficiency, feeding behavior, RUT and accelerometer-based behaviors were analyzed with a mixed general linear model (PROC MIXED, SAS 9.4) with diet, inoculation and diet x inoculation interaction as fixed effects and initial exit velocity as a co-variate. Although steers were randomized into treatments with pre-study exit velocity as a consideration, there were differences between treatments on days 0 and 14 of the study. Therefore, initial exit velocity was used as co-variate to remove any effect of

temperament on dependent variables. This analysis was conducted on the first 28 d of the study prior to inoculation, the 28 d following (28 – 56) inoculation and the 56 d (28 – 84) following inoculation. These time periods were selected to identify the performance and behavior changes imposed by the LY supplementation prior to the inoculation and identify the impact of LY supplementation during and post-disease challenge. Multiple comparisons of least squares means were calculated with the P-diff function within the MIXED procedure.

Results

Initial 28 Days

Results for growth, feed efficiency, BV and meal behavior traits are located in Table 4. Table 5 contains the results for RUT and the accelerometer-based behavior traits. Growth performance and DMI prior to the inoculation were within normal range for the type of cattle and diet fed (Kayser et. al., 2015). There were no significant inoculation x diet interactions detected during the first 28 d period prior to MH challenge.

Initial and final BW were not affected by the dietary treatment, although steers fed LY had a 12.6% reduction ($P < 0.01$) in ADG compared to CON treatment. There were no differences in DMI between the dietary treatments, but when expressed as a percentage of BW, LY-supplemented steers consumed 5.3% less ($P < 0.05$) and had reduced ($P < 0.01$; 0.14 vs. 0.16) G:F relative to the CON treatment. Supplementation with LY reduced ($P < 0.05$) BV frequency 13.6% and increased ($P < 0.01$) TTB by 19

min relative to the CON treatment. Supplementation with LY did not impact any other BV or meal event feeding behavior traits. There were no effects of LY supplementation on the accelerometer-based behaviors (rumination, rest, etc.). There was a tendency for LY to increase ($P < 0.08$) average RUT (39.4 vs 39.3, °C). Prior to being challenged there should have been no differences between inoculation treatments, however steers in the MH inoculation treatment had greater daily DMI Var (2.06 vs. 1.80, $P < 0.05$) and spent 10 min/d more ($P < 0.05$) displaying over activity.

Days 28 to 56

Similar to the initial 28 d of the study, there were no differences between dietary or inoculation treatments for initial and final BW (Table 6). However, post inoculation, steers supplemented with LY displayed a 32.7% increase ($P < 0.02$) in ADG and a 36.3% increase ($P < 0.01$) in G:F compared to the CON steers. There were no dietary treatment differences in BV frequency post-inoculation, although LY supplementation increased ($P < 0.01$) NFI duration by 62 min compared to the CON fed steers. There were no differences between the dietary treatments for any other feeding behavior, accelerometer-based behavior traits or RUT.

Inoculation treatment did not impact final weight or ADG, although MH-challenged steers numerically gained less and had reduced BW at the end of the period study period. Inoculation with MH tended ($P < 0.06$) to reduce DMI and DMI as percent of BW compared to PBS-inoculated steers. Additionally, MH-challenged steers displayed a 43.8% increase ($P < 0.01$) in variability of daily feed consumption.

Inoculation treatment did not impact the BV or meal behavior traits, however MH-challenged steers exhibited a 12.7% reduction ($P < 0.02$) in eating rate compared to the PBS-challenged steers. During the 4 wk post-inoculation period there were no differences detected in any of the accelerometer based behavior traits due to inoculation treatment (Table 7).

Steers inoculated with MH had increased ($P < 0.02$) maximal RUT of 40.5 compared to 40.3 °C of the PBS-challenged steers. There were no other main effect differences in RUT measures; however, an inoculation x diet interaction was detected for minimum RUT ($P < 0.01$) and RUT SD ($P < 0.01$; Fig. 5). The MH-CON steers had greater ($P < 0.05$) minimum RUT than the PBS-CON and MH-LY steers with the PBS-LY steers being intermediate. The PBS-LY steers had the least variation in RUT, which was significantly ($P < 0.05$) less than the PBS-CON and MH-LY steer with the MH-CON steers being intermediate.

Days 28 to 84

Steers were fed their experimental diets continuously from days 0 through 56, therefore the final time period spans the final 4 wk of supplementation and 4 wk of when steers were both fed the CON diet. There were no diet x inoculation interactions detected for any of the growth, feed efficiency or feeding behavior traits (Table 8). Initial and final BW were not different between the diet or inoculation treatments. Although, the final BW of steers supplemented with LY was numerically 13 kg greater than CON treatment and MH-challenged steers were numerically 17 kg lighter than the PBS-

challenged steers. Steers supplemented with LY continued to have an increased ($P < 0.05$) ADG (1.56 vs. 1.33, kg/d) throughout the duration of the study compared to the CON steers. Similarly to the 28-56 d period, LY improved ($P < 0.02$) G:F by 15.4%, relative to the CON treatment. Supplementation with LY increased ($P < 0.05$) NFI duration by 25 min/d, but did not impact any other BV or meal feeding behavior traits. Effects of the MH challenge persisted throughout the duration of the study, with MH-challenged steers tending to have reduced ($P < 0.07$) DMI and DMI as a percentage of BW ($P < 0.09$). Relative to PBS-challenged steers, MH-challenged steers displayed greater ($P < 0.01$) variability in daily DMI and reduced ($P < 0.03$) eating rate.

Throughout the last 56 d of the study there were no differences detected between the dietary or inoculation treatments in any of the accelerometer-based behavior traits (Table 9). Supplementation with LY marginally increased ($P < 0.05$) average RUT of 0.1 °C greater than the CON treatment. Inoculation with MH had long term effects on RUT; similar to the 28-56 d time period, MH-challenged steers tended to have greater ($P < 0.08$) maximal RUT compared to the PBS-challenged steers. There were no other main effect differences in RUT between the inoculation and dietary treatments, although significant interactions in minimum RUT ($P < 0.02$) and SD of RUT ($P < 0.01$, Fig. 5) were detected. Steers in the PBS-LY treatment had greater ($P < 0.05$) minimum RUT than PBS-CON and MH-LY treatments. The MH-CON treatment was intermediate and not different from any of the treatments. Steers fed LY that were inoculated with PBS had the least variation in RUT, which was less ($P < 0.05$) than the PBS-CON and MH-

LY treatments. The MH-CON treatment was intermediate and not different from the other treatments.

Discussion

There has been a renewed interest in the use of direct fed microbials (DFM) in beef production. The US Food and Drug Administration (FDA) defines DFM as "a source of live (viable) naturally occurring microorganisms" (Yoon and Stern, 1995). There are two main types of DFM currently in use, bacterial DFM typically populate the small intestine where they competitively exclude other bacteria and yeast cultures, which alter the rumen microbial populations (McAllister et. al., 2011). Proposed modes of action for yeast DFM occur in the rumen where it is believed they favorably alter digestion by modulating acid production, promote growth of desirable microbial populations, enhance ruminal fiber digestion and scavenge oxygen within the rumen (McAllister et al., 2011; Newbold et al., 1996). The goal of utilizing these products is to modify the rumen environment to increase productivity and health of the animal.

This experiment was designed to determine the effects of LY supplementation on growth, feed efficiency, RUT, and behavior, pre- and post-disease challenge. The authors chose the experimental challenge model in lieu of a high-risk calf model because it offered the ability to monitor the animals prior to the challenge. Although, the MH challenge induced behavioral and RUT changes there were no differences in clinical observations of disease and only 1 steer (MH treatment) was treated for BRD. Therefore, this model was considered sub-clinical and explains why the differences between the

inoculation treatments are subtle and lack statistical significance, although steers inoculated with MH had a reduced eating rate and increased minimum RUT. There were tendencies for reduction in DMI and DMI expressed as a percent of BW and these behavior alterations persisted throughout the duration of the study. The effects of this challenge model were similar to that described by Capik et al. (2015), who reported mild signs of depression in challenged calves with only 1 animal displaying clinical symptoms. Furthermore, the observed febrile response was rather transient and did not extend past 12 h for the majority of the animals (Capik et. al., 2015). Theurer et al. (2013) and Corrigan et al. (2007) reported similar results with MH challenge inducing mild clinical signs of disease that did not exceed the treatment threshold and there were no incidences of fever observed past 24 h.

The live yeast evaluated in the current study is a sub-species of *S. cerevisiae* and genetically indistinguishable. However, metabolically and physiologically LY displays very different characteristics in relation to growth, resistance to high temperature and low pH environments (Fietto et. al., 2004). Although there is yet no proven mode of action, LY is believed to be more active in the small intestine relative to *S. cerevisiae*, due to its ability to grow/survive in low pH environments such as the abomasum and distal duodenum (Czerucka et. al., 2007). In human patients LY has been shown to be effective at ameliorating antibiotic-associated (Surawicz et. al., 1989) and traveler's diarrhea (McFarland, 2007).

There are many examples where yeast DFM supplementation has exerted beneficial improvements on milk yield, feed efficiency and growth of cattle, although

results have been inconsistent (McAllister et. al., 2011). Improvements in DMI has been reported in postnatal calves (Lesmeister et. al., 2004; Galvão et. al., 2005; Pinos-Rodríguez et. al., 2008), lactating dairy cows (Desnoyers et. al., 2009) and growing beef cattle (Finck et. al., 2014; Wagner et. al., 2016). Throughout the current study, LY did not alter DMI during any of the time periods. In fact, it nominally reduced (4%) DMI during the first 28 d and nominally increased (2%) DMI 28 and 56 d post-challenge. Similarly, Zinn et al. (1999) and Keyser et al. (2007) reported no differences in DMI between growing steers supplemented with *S. cerevisiae* or LY, respectively. DeVries and Chevaux (2014) and AlZahal et al. (2014) reported no differences in DMI between lactating dairy cows supplemented with *S. cerevisiae* and negative controls. Galvão et al. (2005) conducted an experiment using postnatal calves where they compared negative controls to calves supplemented with LY in the milk replacer, *S. cerevisiae* supplementation in the grain or both LY in the milk and *S. cerevisiae* in the grain. Calves that received *S. cerevisiae* in the grain exhibited improved DMI over the negative controls and LY supplemented calves were intermediate in DMI and similar to control or *S. cerevisiae* supplemented calves. In two meta-analyses that identified the impacts of *S. cerevisiae* supplementation in growing beef cattle (Wagner et. el., 2016; 18 experiments) and milk producing ruminants (Desnoyers et. al., 2009; 157 experiments), both authors reported a 1.0% increase in DMI of animals supplemented with *S. cerevisiae* relative to controls. Therefore, either LY supplementation does not impact DMI or the mean separation/variance structure is such; that a difference could not be identified in a single experiment.

Supplementation with LY during the first 28 d certainly impacted the manner in which steers interacted with feed. The LY treatment had fewer BV events and took longer to react to feed delivery, suggesting perhaps the LY steers were more satiated than their CON counterparts during the first 28 d. These results are contrary to DeVries and Chevaux (2014) who observed that *S. cerevisiae* supplementation increased the frequency of meals, which reduced overall meal size. They concluded that this change in meal patterns was an improvement and would potentially reduce the incidence of sub-acute ruminal acidosis. In a similar study Yuan et al. (2015) observed quadratic effects on feeding behaviors in relation to dose. Prepartum dairy cows supplemented with 30 – 60 g/d of *S. cerevisiae* exhibited increased meal frequency and a reduction in inter-meal interval and meal size. Post-calving these changes were not observed, likely due to the increase in nutrient requirements associated with milk production greatly altering the feeding behaviors. The behavior differences identified during the first 28 d of the current study were not seen post-inoculation. The lack of difference could be due to induced feeding variation associated with the challenge or animals naturally modifying their feeding behavior in response to days on feed. Post-inoculation, steers supplemented with LY exhibited increased NFI duration for both time periods. This time spent not feeding was partially used for rumination. Although, there were no statistical differences between the treatments during both post-inoculation time periods, steers supplemented with LY had increased rumination time. DeVries and Chevaux (2014) reported a tendency for *S. cerevisiae* to increase daily rumination duration by 25 min. Increased rumination with no changes in DMI would lead to improved digestibility of feed and

could potentially explain the observed increased growth post inoculation for the LY treatment.

Initially, supplementation with LY reduced both ADG and G:F, which may be the result of a loss of microbial efficiency due to population shifts in the rumen. However, post-challenge steers supplemented with LY had improved ADG and G:F relative to the CON treatment. This improvement was a result of a decrease in ADG of the CON treatment that was induced by the challenge. Steers fed LY were less affected by the stress of the MH challenge and maintained the same level of production post challenge. Cole et al. (1992) conducted 3 experiments to identify the effects of *S. cerevisiae* on health and performance of newly received cattle and cattle experimentally inoculated with bovine herpes virus-1 (BHV-1). The yeast culture did not significantly affect the health or performance of calves in the receiving studies, although morbid cattle in experiment 2 that were fed yeast required fewer days of antibiotic therapy. When steers were challenged with BHV-1, calves that were fed yeast tended to maintain heavier weights and DMI relative to the control animals. Word et al. (2016) sampled metabolites on beef heifers following a combined BHV-1 and MH challenge. Heifers supplemented with *S. cerevisiae* and *S. cerevisiae* cell wall exhibited increase circulating glucose and decreased blood urea nitrogen. The authors concluded that supplementation with *S. cerevisiae* may modulate energy stores by reducing muscle catabolism to provide energy for the immune response, which has the potential to improve animal performance (Word et. al., 2016). There have been multiple reports illustrating that *S. cerevisiae* supplementation reduces morbidity. Galvão et al. (2005) examined the effects of *S.*

cerevisiae supplementation on Holstein calves who did not receive transfer immunity from their mothers and reported that supplementation reduced the number of days the calves had diarrhea prior to weaning. Supplementation with *S. cerevisiae* also increased pre-weaning ADG, although the differences were not observed post weaning (Galvão et. al., 2005). Zinn et al. (1999) evaluated the *S. cerevisiae* supplementation on shipping-stressed crossbred calves and reported a 48% reduction in morbidity as well as a 44% reduction in days the calves were morbid. However, there were no differences in ADG or feed efficiency (Zinn et. al, 199). Furthermore, Keyser et al. (2007) reported that high risk heifer calves supplemented with LY had reduced morbidity (13.8 vs. 24.0%) compared to the controls. Control heifers were approximately twice (odd ratio = 1.99) as likely to be treated for BRD as were heifers supplemented with LY (Keyser et. al., 2007). In the current study there were no differences in morbidity between the inoculation groups and no interactions present between dietary and inoculation treatments. However, the increase in ADG and G:F post challenge due to LY supplementation may be the result of improved immune capacity to combat sub-clinical respiratory disease, which led to greater energetic efficiency.

The accelerometer-based behaviors were not impacted by diet or inoculation, with the exception of over activity differences between the inoculation treatment groups prior inoculation. Over activity is an indirect measure of estrus and since these were steers this is most likely an error. There were no differences in ingestion time between the inoculation treatments and likewise there was no difference in BV or meal duration measured by the GrowSafe system. Rumination has been shown to decrease during

respiratory disease (Schroeder and Moys, 1954), acute clinical mastitis (Siivonen et. al., 2011) and post acidosis challenge (DeVries et. al., 2009). However, Thorup et al. (2016) reported no differences in rumination when animals were lame, furthermore the variation of time spent ruminating per day within animal was < 15% but up to 50% across animals. DeVries and Chevaux (2014) reported a 25 min increase in rumination duration due to supplementation with *S. cerevisiae*. The accelerometer-based behavior system was validated against the Institut national de la recherche agronomique (INRA; Paris, FR) reference method. The INRA method consists of combining data from automatic electronic feed bunks, which assign feed to an individual animal and bite meters (Delagarde and Lemonnier, 2015). The bite meter consists of a foam filled balloon which is placed on the lower jaw and connected to a pressure recorder by a silicone tube (Baumont et. al., 2004). Feeding and rumination times are identified by comparing the behaviors measured by each system. Delagarde and Lemonnier, 2015 concluded that the accelerometer-based system was 89 and 90% accurate for feeding and rumination, respectively. Although, they estimated that the system would only be able to detect a 20% difference in intra-animal day to day variation. Therefore, in the current study either the challenge model or LY supplementation did not alter rumination, or the magnitude of the difference between groups was not great enough to be detected by the collars.

Supplementation with *S. cerevisiae* has been shown to impact the ruminal microbial population in a manner that favorably alters fiber digestion and modulates acid production (McAllister et. al., 2011). Desnoyers et al. (2009) reported that *S. cerevisiae*

supplementation increased rumen pH, volatile fatty acid concentration and tended to decrease lactic acid concentration. AlZahal et al. (2008) investigated the relationship between rumen pH and RUT in an effort to detect subacute ruminal acidosis (SARA) using RUT. Six rumen-fistulated lactating Holstein cows were allocated to either a control or SARA challenge diet. Cows fed the SARA diet spent more time below ruminal pH 5.6 and greater time above 39.2 °C and the ruminal pH nadir exhibited a negative relationship ($R^2 = 0.77$) with the corresponding RUT (AlZahal et. al., 2008). Cho et al. (2014) in an effort relieve heat stress in Hanwoo steers, discovered that supplementation with an additive containing 25% yeast culture effectively reduced RUT and rectal temperature, although there were no differences in pH. DeVries and Chevaux (2014) reported that *S. cerevisiae* supplementation reduced mean RUT (38.4 vs. 38.5 °C) in lactating Holsteins and reduced the time (min/d) that RUT was greater than 39 °C. In the current study, supplementation with LY tended to increase RUT during the initial 28 d and was significantly greater from days 28 through 84. Even though the mean separation was equal for all time periods there was no significance between days 28 through 56, which could be a result of increased variance associated with a febrile response induced by MH inoculation. This slight increase in RUT could potentially be indicative of improved digestibility and greater concentration of volatile fatty acids in the rumen, which would partially explain the divergence in ADG of the steers fed LY. Steers in the MH inoculation had increased maximum RUT following the challenge and had a tendency for increased RUT throughout the duration of the study. Timsit et al. (2011) utilized thermoboluses to monitor BRD in young bulls and found that RUT

increased prior to rectal temperature and clinical signs of BRD. Furthermore, during RUT hyperthermia episodes, the correlation between RUT and the coinciding rectal temperature was $R^2 = 0.82$ (Timsit et. al., 2011). Although the MH challenge was sub-clinical, these increases in RUT provide evidence that the challenge effectively stimulated an immune response. The interactions present in minimum RUT and RUT SD for the 2 post-inoculation periods are difficult to interpret. Minimum RUT is not different between PBS-CON and MH-LY or MH-CON and PBS-LY treatments for either time period, although the multiple comparisons are different from days 28-56 relative to days 28-84 the pattern between the treatments remains the same. The multiple comparisons for RUT SD are analogous to RUT minimum, where the PBS-CON and MH-LY treatments behave similarly. Although statistically significant, differences among treatments are negligible (0.03 °C) and likely not biologically relevant. Results from this study show that the experimental MH challenge was effective at stimulating a febrile response and that the thermoboluses were sufficiently accurate to detect that response.

Conclusion

The experimental MH challenge model induced behavioral and physiologic changes in growing beef steers without creating gross signs of clinical disease. Supplementing growing steers with LY improved ADG and feed efficiency post experimental challenge and these improvements continued after cessation of supplementation throughout the duration of the study. These results suggest that LY

supplementation improves growth and efficiency during times when the animal is stressed and/or the immune system is compromised. Research needs to be further conducted to understand the negative effects on growth and efficiency due to LY supplementation prior to challenge.

CHAPTER 4

EFFECTS OF COMBINED VIRAL-BACTERIAL CHALLENGE WITH OR WITHOUT SUPPLEMENTATION OF *Saccharomyces cerevisiae boulardii* STRAIN I-1079 ON IMMUNE UPREGULATION AND DMI IN BEEF HEIFERS

Introduction

Experiment objectives were to determine if live yeast (LY) supplementation would impact DMI, body weight (BW), immune and febrile responses to a viral-bacterial (VB) respiratory challenge. Thirty-eight crossbred Angus heifers (initial BW = 230 ± 16.4 kg) were allocated to treatments in a 2 X 2 factorial arrangement: Factor 1 = roughage-based diet with or without LY (*Saccharomyces cerevisiae boulardii* I-1079, 62.5 g/hd/d), Factor 2 = VB, intranasal administration of bovine herpesvirus-1 (BHV-1, 2 x 10^8, PFU) and broncho-selective endoscopic inoculation with *Mannheimia haemolytica* (MH, 5.4 x 10^{10}, CFU) 3 d after or intranasal saline administration followed by inoculation with phosphate buffer solution (PBS). Heifers were randomized by MH and BHV-1 titer status, and fed their respective diets for 27 d prior to VB challenge on day 0. Animals were housed in pens by treatment (2 pens / treatment) and group-fed using electronic feedbunks. Thermo-boluses (Medria) measured rumen temperature (RUT) at 5-min intervals and rectal temperature was measured on days 0, 3-8, 10, 13 and 15. Whole blood samples were collected via jugular venipuncture on days -13, -6, 0, 3-8, 10, 13 and 15 for complete blood count analysis. Data were analyzed using repeated measures in the mixed procedure of SAS with fixed effects of day, diet, inoculation and

their interactions. Animals fed LY exhibited a 16% increase ($P = 0.02$) in neutrophils relative to controls. There were significant diet x inoculation x day interactions for monocytes and haptoglobin. The VB-LY treatment had the greatest ($P < 0.05$) concentration of monocytes on day 4, followed by the VB-CON treatment which was greater ($P < 0.05$) than both the PBS treatments. Haptoglobin was greatest ($P < 0.02$) for the VB-CON treatment on day 5, and the VB-LY treatment was greater ($P <0.05$) than both of the PBS treatments. Heifers supplemented with LY had less ($P < 0.05$) overall haptoglobin production than the CON treatment. Viral-bacterial challenge produced nasal lesions that increased ($P < 0.01$) with day, reaching a nadir on day 6 with approximately 70% of the naris covered with plaques, and increased ($P < 0.05$) neutrophils on days 3 to 5 relative to PBS. Viral-bacterial challenge increased RUT ($P < 0.05$) on days 2 to 7, but decreased RUT on days 9 to 12 relative to PBS. Rectal temperature was greater for VB heifers ($P < 0.05$) on days 0, 3 to 6 and rectal temperature of PBS heifers never exceeded 40 °C. The increase in rectal temperature on day 0 was most likely due to increased ambient temperature at time of challenge, as VB heifers were processed after the PBS heifers to avoid contamination. The VB challenge was effective at stimulating immune responses, and RUT was more sensitive than rectal temperature for measuring febrile responses. These results indicate that prior LY supplementation altered the leukogram in response to VB challenge, suggestive of increased innate immune response.

Bovine respiratory disease (BRD) complex is one of the primary economic and animal welfare challenges facing beef producers (Griffin, 2014; Rose-Dye et al., 2011).

Despite efforts to reduce mortality and morbidity through vaccination, antimicrobial therapy and management strategies, mortality due to BRD in the feeding period is increasing and respiratory disease is the leading cause of death for cattle in the United States (Engler et al., 2014; USDA, 2015). The BRD complex is multifactorial and includes bacterial and viral pathogens that are often co-infected, which results in increased severity of disease (Griffin et al., 2010).

Bovine herpes virus-1 (BHV-1) is a viral pathogen within the BRD complex that leads to upper respiratory tract disorders and exerts immuno-suppressive effects, which increases susceptibility to secondary bacterial infection (Jones and Chowdhury, 2007). *Mannheimia haemolytica* (MH) is the most prevalent pathogen associated with BRD (Smith, 2015). Although considered commensal it is also opportunistic and is the most common isolate found in feedlot cattle with fatal fibrinous bronchopneumonia (Ackerman and Brogden, 2000).

The US Food and Drug Administration's (FDA) recent changes to the Veterinary Feed Directive Final Rule (2016) expresses interest in antibiotic alternative solutions for treatment and prevention of BRD. This supports consumer perception against antibiotic use in livestock production, evident by the rise in demand for natural and organic protein sources. In order for livestock producers to maintain a social license, while sustainably producing beef, antibiotic alternatives are a prerequisite. Direct-fed microbials (DFM) are potential antibiotic alternatives, which have been shown to be effective at reducing morbidity in shipped stressed calves (Zinn et. al., 1999). *Saccharomyces cerevisiae boulardii* (LY) is one of the most widely studied microorganisms due to its application

in human patients to combat enteric diarrhea, however little research has been conducted in ruminants (Łukaszewicz, 2012).

Based on this need for antibiotic alternatives, the objectives of this experiment were two-fold. First, to evaluate if dietary supplementation with LY would influence immune response following the viral-bacterial challenge and potentially ameliorate the detrimental effects. Second, to develop a viral-bacterial challenge model consisting of intranasal inoculation of BHV-1 followed by endoscopic inoculation of MH that would allow us to study immune and behavior changes associated with the acute response to BRD. Furthermore, characterize alterations of the hemogram, acute phase proteins and continuously measured physiologic and behavior responses imposed by the challenge.

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC # 2015-0379) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2015-068).

Animals

A total of 38 Angus crossbred heifers (initial BW = 230 ± 16.4 kg) from the McGregor cattle herd belonging to Texas A&M University were used in this study. Initially, 40 heifers were enrolled with 10 hd per treatment arrangement but 2 heifers were removed from the analysis due to lameness. All animals were considered clinically

healthy based upon daily observations for 27 d prior to challenge and were seronegative for MH determined by a serum sample collected prior to study enrollment. Serum titers were evaluated for exposure to BHV-1, and heifers that fell within the middle of the distribution were selected for use in the study. Furthermore, all animals were confirmed negative for persistently infected bovine viral diarrhea virus (BVDV), through collection of an ear notch prior to study commencement, which was analyzed with the BVD antigen-capture ELISA (BVD-Ag ELISA).

Experimental Design and Treatment Arrangements

Heifers were stratified by initial BW, MH and BHV-1 titer dilution, exit velocity and pre-study ADG, and randomly assigned to 1 of 4 treatments arranged in a 2 x 2 factorial array. Factor 1 consisted of a roughage-based diet without (CON) or with added LY (*Saccharomyces cerevisiae boulardii* strain I-1079 at 62.5 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition, Milwaukee, WI). Factor 2 consisted of intranasal inoculation with BHV-1 followed 3 d after with bronchoselective endoscopic inoculation with MH (VB) or intranasal inoculation with saline followed 3 d later with bronchoselective endoscopic inoculation with phosphate buffer solution (PBS). Therefore, the 4 treatments arrangements were: (n = 10) VB-CON, (n = 10) VB-LY, (n = 9) PBS-CON, and (n = 9) PBS-LY.

This experiment lasted 22 d, and was within a larger 84 d growth and performance study. Throughout the study all animals were group housed in 8 pens equipped with electronic feedbunks (GrowSafe) at Texas A&M University's McGregor

Research Center in McGregor, TX. Heifers were segregated in pens by treatment arrangement and replicated twice. To ensure that PBS treatment heifers would not be contaminated with BHV-1, the inoculation treatments were penned on opposite sides of a barn with the BHV-1 treatment on the prevailing downwind side. Heifers were offered feed *ab libitum*, which was provided daily at 0700 h. The diet (DM basis) contained 36.5% dry rolled corn, 26% corn dried distillers grains, 30% chopped alfalfa hay, 5% molasses and 2.5% dry mineral. Targeted intake of LY in this study was 62.5 g/hd daily which was premeasured and hand mixed in the diet prior to delivery. Similarly, 62.5 g/hd of an isonitrogenous isocaloric placebo was included in the control diet daily. Feeders were blinded to dietary treatments, and the LY and CON diets were analyzed weekly for LY strain I-1079. The LY diet always contained colony forming units (CFU) at or above the prescribed level of CFU for this study of 2.5×10^{10} per day. Throughout the study no I-1079 or wild yeast colonies were detected in the CON diet.

Prior to day 0, the inoculum for the viral-bacterial challenge was prepared. Briefly, the Cooper strain of BHV-1 was used in this study, and was originally obtained from the United States Department of Agriculture's (USDA), Center for Veterinary Biologics (CVB) in Ames, IA. The virus was propagated by inoculating 12, 850 cm^2 roller bottles of Madin Darby bovine kidney (MDBK) cells. Cells were grown in Eagles minimum essential medium (EMEM) with Earles basic salt and 10% fetal bovine serum (FBS). Growth medium was poured off and the BHV-1 inoculated at 10-1 dilution onto roller bottles and allowed to adsorb for 1 h at 36 ± 2 °C in 5 ± 1% CO_2. After which, EMEM with Earles salts and 2% FBS was added and roller bottles were returned to 36 ±

2 °C in 5 ± 1% CO_2 until cytopathic effect was observed to be approximately 90%. Virus was harvested by freeze/thaw, and the fluid containing the virus was centrifuged at low speed (500 x g for 20 min) harvested and stored at -70 °C. Virus was distended in media to achieve the desired concentration of 1 x 10^8 plaque forming units (PFU) per mL.

The MH inoculum was prepared as described by Mosier et al. (1995). Briefly, MH serotype A1 was grown on trypticase soy agar containing 5% sheep blood for 18 h at 37 °C in 7% CO_2. Colonies were inoculated into brain-heart infusion broth and incubated for 16 to 18 h at 37 °C with aeration. The bacteria were then centrifuged at 3,000 × g for 15 min at 4 °C and washed with PBS twice. After the second wash, the bacteria were centrifuged as before, and the pellet was re-suspended in PBS at a final concentration of 5.4 × 10^{10} CFU/10-mL dose. After preparation, the inoculum was placed on ice in a dark cooler and transported to the site of inoculation (approximately 174 km).

On day 0, 1-mL of either BHV-1 at 1 x 10^8 PFU/mL or saline was aerosolized into each naris with a 3 mL syringe fitted with and intranasal mucosal atomization device (MAD Nasal; Teleflex, Morrisville, NC). Heifers in the PBS treatment were inoculated prior to heifers in the VB treatment and the chute and processing area were disinfected after BHV-1 inoculation. On day 3 all heifers were brought back to the process facility and endoscopically inoculated with MH or PBS. In order to avoid any chance of PBS animals receiving MH via contamination of the instruments used for inoculation, the PBS treatment group was inoculated prior to the MH treatment group. The inoculations were performed with an endoscope as described by Theurer et al.

(2013). Heifers were captured in a standard squeeze chute and their heads were restrained with a halter specifically designed for cattle. An endoscope 1 m in length was inserted into the ventral meatus of one nostril and passed into the trachea to the level of the right apical lung lobe bronchi allowing visualization of the opening. A sterile bronchoalveolar lavage tube was inserted into the endoscope portal and passed until the tip of the lavage tube was visible emerging from the endoscope. The lavage tube was advanced another 1 to 2 cm into the opening of the right apical lung lobe bronchi. Once the lavage tube was in place, heifers in the PBS treatment group were administered 10 mL of PBS followed by a 60 mL flush of PBS for a total of 70 mL. Following inoculation of PBS animals, the endoscope was disinfected with chlorhexidine solution and rinsed with saline. Subsequently, heifers in the MH treatment groups were challenged with 10 mL of *M. haemolytica* serotype A1 at 5.4×10^{10} CFU/mL followed by 60 mL of PBS for a total of 70 mL. No adverse effects due to the inoculation procedure were observed for either treatment.

Data Collection

Temperature Monitoring, Nasal lesion and Clinical Illness Scoring. Rectal temperatures were recorded using a digital thermometer (Cooper TM99A, Cooper-Atkins Corporation, Middlefield, CT) on days -6, 0, 3, 4, 5, 6, 8, 10, 13 and 15. In addition, radiofrequency biothermal boluses (ThermoBolus, Medria, Châteauborg, France) were inserted into the rumen of all heifers. The ThermoBolus continuously recorded reticulo-rumen temperature (RUT) at 5-min intervals. A proprietary algorithm

was used to remove variation in RUT due to drinking events. Summary statistics of RUT were computed on a daily basis for the duration of the experiment. After inoculation with BHV-1 the mucosa of the nares was observed to quantify the formation of BHV-1 plaques. Nasal lesions were subjectively quantified by 1 observer based on the percentage of visible naris covered with plaques in 10% increments. This was done until all nasal plaques were resolved (d 13). Heifers were monitored by two experienced evaluators twice daily for the duration of the experiment for clinical signs consistent with BRD. The visual evaluation employed in the experiment has been described in detail by Step et al. (2008). The criteria includes signs of depression, inappetence and respiratory distress. The evaluators assigned a severity score of 1 to 4, where 1 was assigned for mild, 2 for moderate, 3 for severe and 4 for moribund. Heifers receiving a 3 or greater were pulled from the pen and given a full medical evaluation. Rectal temperature was measured during the medical evaluation and if exceeded 40.5 °C anti-microbial therapy was administered. All heifers were returned to their home pen after the evaluation. Temperature readings, BW and treatments were recorded for every animal that was examined for clinical signs consistent with BRD. The first treatment administered to heifers suffering from BRD was tulathromycin (Draxxin, Zoetis, Parsippany, NJ) at 2.5 mg/kg BW. If the initial treatment was ineffective and the heifers were still suffering after 7 d, ceftiofur hydrochloride (Excenel, Zoetis, Parsippany, NJ) was administered at 2.2 mg/kg BW.

Serum Haptoglobin and Cortisol. Blood samples (10 mL; Vacutainer with no additive, Becton, Dickson and Company, Franklin Lakes, NJ) were collected via jugular venipuncture with an 18-gauge needle on days -6, 0, 3, 4, 5, 6, 8, 10, 13 and 15, relative to BHV-1 challenge. After collection samples were immediately placed on ice. To harvest serum, samples were centrifuged at 3,000 x g for 20 min at 20 °C, and stored in duplicate aliquots at -20 °C until subsequent analysis. Haptoglobin was measured with a commercial, bovine-specific sandwich ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR). Furthermore, the concentration was determined by the average of duplicate unknown samples compared with a standard curve (four-parameter logistic) generated from known concentrations of bovine haptoglobin using Assay Zap software (Biosoft, Cambridge, UK). The haptoglobin analysis had an interassay CV of 3.81%. Serum concentrations of cortisol were determined as described by Littlejohn et al. (2016) and Burdick et al. (2009). A solid phase radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX) using antiserum-coated tubes were prepared according to the manufacturer's directions. Serum cortisol concentrations were determined by the average of duplicate unknown samples compared with a standard curve generated from known concentrations of cortisol using Assay Zap software (Biosoft, Cambridge, UK). The minimum detectable cortisol concentration for this assay was 1.02 ng/mL, and the interassay CV was 11.4%.

Hemogram. Blood samples (7 mL; EDTA, Becton, Dickson and Company, Franklin Lakes, NJ) were collected via jugular venipuncture with an 18-gauge needle on days -6,

0, 3, 4, 5, 6, 8, 10, 13 and 15, relative to BHV-1 challenge. Samples were immediately submitted to a commercial lab (Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX) for total leukocyte, erythrocyte, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets. Blood counts were performed with an automated hemocytometer (ADVIA 120, Siemens Healthcare Diagnostics, Tarrytown, NY) using the factory installed cattle setting (ADVIA 120 Multispecies System Software, Version 2.206 MS, Siemens Healthcare Diagnostics). The hemocytometer counts leukocytes, erythrocytes and platelets by optical scatter and fluorescence. Hemoglobin concentration is determined by the cyano-methemoglobin technique. Differential leukocyte percentages were determined by counting cells on modified blood smears and absolute counts were calculated using the total leukocyte count from the hemocytometer.

Feed Intake. All pens were equipped with electronic feedbunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake on an individual-animal basis. The GrowSafe system consisted of feed bunks equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of EID tags. Assigned feed disappearance (AFD) rates were computed daily for each feed bunk to assess data quality. If the AFD < 95%, the day's data was omitted from the analysis. Of the 176 (22 d x 8 pens) possible collection days 14 were omitted due to inadequate collection. Data was omitted for 4 d (-3, -2, 4 and 8)

from Pen 1, 5 d (2, 4, 10, 11 and 12) from Pen 3, 3 d (11, 12, 13) from Pen 5 and 2 d (12 and 13) from Pen 8. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance during each bunk visit event. A subroutine of the GrowSafe 6000E software (Process Feed Intakes) was used to compute daily feed intake.

Statistical Analysis

This experiment was designed as a randomized complete block with a 2 x 2 factorial treatment arrangement with animal serving as the experimental unit. Temperature measures, feed intake, haptoglobin, cortisol and hemogram measurements were analyzed using the MIXED procedure (SAS 9.4, SAS Institute Inc., Cary, NC) with an autoregressive covariance structure. The model for all variables included the main effects of diet, inoculation, day and all possible interactions. Average daily gain measured during the 27 d prior to challenge was used as a covariate for all analyses. Although heifers were randomized into treatments with pre-study ADG (d -58 through -31) as a consideration, there were differences in ADG between treatments measured from days -27 through 0 of the study. Therefore, initial ADG was used as co-variate to remove any effects of ADG on dependent variables. When there was dependent variable x day interaction present ($P \leq 0.05$) the SLICE output option was used to identify differences within day. In the event a diet x inoculation or diet x inoculation x day interaction was present ($P \leq 0.05$), least squares means were separated using the PDIFF multiple comparison test. Growth rates for individual heifers were modeled by linear

regression of BW measurements on study day using PROC GLM (SAS, 9.4). The regression coefficients were used to compute ADG, initial BW and final BW.

Results

Initial (day -6) and final (day 15) BW were not impacted by the inoculation or dietary treatments, and there were no interactions detected for BW. The dietary treatment did not impact ADG, and there was no diet x inoculation treatment interaction detected for ADG. However, VB-challenged heifers gained 0.31 kg/d, which was less ($P < 0.01$) than PBS-challenged heifers who gained 1.21 kg/d. Furthermore, an inoculation x day interaction ($P < 0.01$) was detected for BW (Fig. 6); however, mean separation between inoculation treatments were not significant within day. Although, caution should be used when interpreting these results because ADG and BW were measured over a relatively short 22 d time-period. Dietary treatment did not impact DMI and a diet x day interaction was not detected. However, there was an inoculation x day interaction ($P < 0.01$) for DMI, with VB heifers having reduced DMI on days 3, 4, 5, 6 and 7 (Fig. 6), compared to PBS-inoculated heifers.

There were no differences in clinical illness scores between the inoculation treatments. In fact, only two heifers (VB treatment) received a clinical illness score ≥ 3. However, when given a medical evaluation they did not have a rectal temperature > 40.5, and therefore were not treated. There were no naturally occurring cases of BRD, re-pulls due to challenge or mortalities during the study. At the time of BHV-1 administration, the mucosal surface of naris was inspected and no lesions were present.

No lesions were observed in the PBS heifers at any time and there were no differences in lesion prevalence due to diet. However, there was a significant (Fig. 7; $P < 0.01$) inoculation x day interaction for nasal lesions. Heifers inoculated with BHV-1 had greater ($P < 0.01$) prevalence of nasal lesions from days 3 to 11, reaching a nadir on day 6 with 70% of the visible nares covered in plaques. Figure 8 is a photographic presentation of the formation of nasal lesions for 1 heifer within the VB treatment, and is representative of the observed nasal lesions during the VB challenge.

Dietary treatment did not impact rectal temperature or RUT, and there were no diet x day interactions detected for either rectal temperature or RUT. Although, there was an inoculation x day interaction ($P < 0.01$) detected for rectal temperature, with VB-challenged heifers having greater rectal temperature on days 0, 3, 4, 5, and 6. Similarly, there was an inoculation x day interaction ($P < 0.01$) detected for RUT, with VB-challenged heifers exhibiting greater RUT on days 2, 3, 4, 5, 6, and 7, reaching a nadir on day 4 with an average RUT of 41.0 °C. Heifers in the VB challenged exhibited decreased RUT on days 9, 10, 11 and 12, relative to PBS heifers.

Hemogram least square means for the diet and inoculation treatments as well as *P*-values for all possible interactions are reported in Table 10. Heifers supplemented with LY had a 14.1% greater ($P < 0.02$) serum concentration of neutrophils compared to the CON heifers. There was a diet x inoculation x day interaction ($P < 0.01$) detected for monocyte concentration (Fig. 9 and Table 11). The day of endobronchial inoculation (d 3), heifers in the VB-LY treatment had the greatest ($P < 0.05$) concentration of circulating monocytes at 1.93 k cells/μL. The VB-CON treatment was less than the VB-

LY treatment, although greater ($P < 0.05$; 1.35 k cells/μL) than both the PBS-LY and PBS-CON, which were not different from each other (0.50 and 0.82 k cells/μL; respectively). On day 5 the VB-CON and VB-LY treatments were greater ($P < 0.05$) than the PBS-CON treatment, and PBS-LY was intermediate and not different from other treatments. On day 6 the VB treatments were greater ($P < 0.05$) than both PBS treatments. On day 8 the VB-LY was greater ($P < 0.05$; 0.82 k cells/μL) than the PBS-CON (0.43 k cells/μL) treatment and VB-CON and PBS-LY were intermediate and not different from other treatments.

Similar to monocytes, a diet x inoculation x day interaction ($P < 0.02$) was detected for haptoglobin concentration (Fig. 10 and Table 12). Following inoculation, VB-challenged heifers had rapid increases in circulating haptoglobin concentrations. Heifers in the PBS treatment never had circulating haptoglobin concentrations > 0.15 mg/dL. On day 4, heifers in the VB-CON treatment had the greatest ($P < 0.05$) haptoglobin concentration of 50.2 mg/dL, with the VB-LY treatment intermediate at 15.1 mg/dL. Both VB treatments were greater ($P < 0.05$) than the PBS treatments whose circulating haptoglobin concentrations were ≤ 0.01 mg/dL. On day 6 VB-LY and VB-CON had haptoglobin concentrations of 35.0 and 25.1 mg/dl, respectively, which were greater than the PBS treatments. The VB treatments had slightly elevated haptoglobin concentrations on day 8; although they were not different from the PBS treatment. By day 10, the VB treatments had returned to pre-inoculation levels of circulating haptoglobin concentration.

Leukogram constituents that were significantly ($P < 0.05$) impacted by day and inoculation are presented in Figure 11. Leukocyte count was greater ($P < 0.05$) for VB-challenged heifers on days 4, 5 and 15 relative to PBS-challenged heifers. Inoculation with the VB challenge reduced ($P < 0.05$) lymphocytes on days 4, 8, 10 and 15 relative to the PBS heifers. Similar to leukocytes, neutrophils rapidly increased in VB-challenged heifers from days 3 to 5, reaching a nadir on day 4. Neutrophils were also increased ($P < 0.05$) in the VB heifers on days 10, 13 and 15, compared to the PBS-challenged heifers although the magnitude of mean separation was less.

Alterations to the erythron due to the significant ($P < 0.01$) interaction of day x inoculation are presented in Figure 12. Even though the day x inoculation interaction for platelets was significant ($P < 0.01$) the results are not presented, due to there being no differences between the treatments on a given day. Total erythrocyte count was reduced ($P < 0.05$) on days 8, 10, 13 and 15, for VB-challenged heifers compared to the PBS-challenged heifers. Hemoglobin was increased ($P < 0.05$) for VB-challenged heifers on day 3 and decreased ($P < 0.05$) on days 10 and 13, compared to the PBS-challenged heifers. Heifers in the VB challenge exhibited reduced hematocrit on days 6, 8, 10, 13 and 15, and increased MCHC on days 4, 5, 6 and 15 compared to PBS-challenged heifers.

Discussion

Although previous reports have utilized a similar (BHV-1 + MH) combined viral-bacterial challenge model, typically the MH is delivered intra-tracheally or

aerosolized (Word et al., 2016; Stabel et al., 1993; Jericho and Langford, 1978). In the current study MH was delivered endobronchially and the only other example in the literature was performed on Holstein calves ≤ 5 months of age (Narita et al., 2000). Therefore, our objectives were to evaluate the challenge model on animals that would be typical of a feedyard placement in the United States, and characterize physiological and behavioral effects. Furthermore, to evaluate if prior supplementation of LY would ameliorate the negative effects from the challenge model on physiologic, immune and behavioral responses.

Common clinical signs associated with naturally occurring BRD include pyrexia, lethargy, anorexia and dyspnea (Duff and Galyean, 2007). Although, the current challenge model did not create gross signs of clinical disease, VB-challenged heifers had marked reductions in DMI following MH inoculation. Reduced feed intake has been well documented in BRD morbidity studies (Sowell et al., 1999, Quimby et al., 2001, Hutcheson and Cole, 1986 and Galyean and Hubbert, 1995). Theurer et al. (2013), reported decreases in duration of time calves spent at a hay feeder that were similarly inoculated with MH. Although fluctuations in bunk visit duration are not equal to DMI, the two measures are moderately correlated (0.37 – 0.52; Kayser and Hill, 2013), and the reduction of duration likely resulted in decreased feed intake. Interestingly, inoculation with BHV-1 did not impact DMI on days 0 to 2, although, this result is in agreement with Reffett et al. (1988) and Stabel et al. (1993). Both studies administered a BHV-1 challenge to Holstein calves and reported no differences in feed intake following inoculation. Similarly, Burciaga-Robles et al., (2010) reported no differences in DMI

between steers exposed to a BVD persistently infected steer and negative controls. In contrast to these reports, Cole et al. (1992) reported decreases in daily feed intake in steers that were challenged with BHV-1. However, the impact of the challenge did not appear to reduce feed intake until 3 d after administration. The impact that BHV-1 challenge exerts on DMI is unclear; in the current study we are only able to evaluate the time between the inoculations in which there was no difference. The reduced DMI resulting from the VB challenge was synonymous with naturally occurring BRD cases, which illustrates the effectiveness of the model.

Clinical symptoms of the respiratory form of BHV-1 share commonality with other pathogens in the BRD complex such as; hyperthermia, anorexia, coughing, nasal discharge and excess salivation (Jones and Chowdury, 2007). However, unique to BHV-1 is the formation of plaques on the mucosa of the nares and trachea (Yates, 1982). Furthermore, McKercher et al. (1957) reported that naturally occurring and experimentally induced BHV-1 lesions are similar; although, experimentally induced BHV-1 cases had increased severity of rhinitis and decreased tracheitis relative to naturally occurring cases (Yates, 1982). In the current study, BHV-1 inoculation created characteristic plaques and erosions on the mucosal surface of the nares, as well as localized inflammation of the nares (Gershwin et al., 2015). Similarly, Word et al. (2016) reported nasal lesion formation following inoculation with BHV-1, and previous supplementation with live yeast and yeast cell wall tended to decrease nasal lesions scores (2.5 vs 3.2, respectively). In the current study supplementation with LY did not impact lesion formation ($P = 0.87$).

Body temperature, often measured rectally is frequently used as a diagnosis for BRD and typically the only objective measurement. The VB challenge induced hyperthermia as measured by both rectal temperature and RUT. Increases in core body temperature due to BHV-1 were observed in RUT beginning on day 2 and on day 3 for rectal temperature. It is possible that rectal temperature increased prior to day 3; however that was earliest rectal temperature was measured after inoculation. A similar febrile response was reported by Cole et al. (1992), where rectal temperature increased as a result of BHV-1 inoculation beginning on day 3, although rectal temperature only exceeded 40 °C for 24 h. Rose-Dye et al. (2011), reported no change in rectal temperature or RUT due to a 72 h exposure with a BVD persistently infected calf; however, inoculation with MH stimulated an almost immediate increase in both rectal temperature and RUT. Increased core body temperature due to experimental MH challenge is fairly repeatable with durations of elevated body temperature lasting between 24 and 36 h (Theurer et al., 2013; Hanzlicek et al., 2010; Burciaga-Robles et al., 2010b; Corrigan et al., 2007; Capik et al., 2015). In the current study increases in core body temperature were observed for longer durations than in the single pathogen challenge studies. Heifers in the VB challenge had greater RUT for 6 d and rectal temperature for 4 d, relative to the PBS treatment. It is impossible to determine if the combination of pathogens created an additive effect due to the design of the current study; however, when these results are compared to previous single pathogen challenges, the magnitude and duration of the febrile response would suggest so. The increased rectal temperature of VB heifers the day of inoculation was most likely to due to

increased ambient temperature at the time of inoculation as VB heifers were processed after the PBS heifers to reduce the potential of contamination. Interestingly, there was no difference between the treatments on day 0 in RUT, suggesting that RUT is more accurate at monitoring core temperature. Rumen temperature was continuously measured on 5-min intervals; therefore, a daily estimation is derived from 288 measurements while the estimation of rectal temperature consists of 1 measurement. There is inherently more error in the rectal temperature measurement compared to RUT and is more likely to be affected by environment. Hanzlicek et al. (2010) measured rectal temperature 3 times daily on beef steers experimentally challenged with MH. They reported that the 3 measurements were statistically different from each other and was greatest (morning = 39.6, noon = 39.4 and evening = 40.2 °C) in the early evening. These results reinforce that the VB challenge effectively stimulated an immune response and suggest that RUT may be more accurate than rectal temperaure for identifying febrile animals.

Heifers in the VB challenge had increased leukocyte concentrations that would be classically associated with upregulation of the innate immune system due to an acute infection. Interestingly the leukogram appears to have only been impacted by the MH inoculation. With the exception of neutrophils, there were no differences in any of the leukocyte or erythrocyte concentrations on day 3. However, the day following MH inoculation (d 4) almost all of the variables were different between the challenge treatments. Monocytes were increased the day following MH inoculation and were greater for the heifers fed LY. The impact of experimental challenge on monocytes is

inconclusive. Similar to the current study, Corrigan et al. (2007) reported an increase in monocytes following inoculation with MH. However, Hanzlicek et al. (2010) reported no difference in monocytes resulting from MH challenge. Furthermore, Burciaga-Robles et al. (2010b) and Gånheim et al. (2005) reported no differences in calves challenged with BVD, MH or a combination of both. Monocytes are macrophages in the circulatory system which are transported to tissues during infection or inflammation, and are the major phagocyte population resident in normal tissues at homeostasis (Murphy and Weaver, 2017). The increase in monocytes following challenge suggests that the model effectively stimulated an upregulation of the innate immune system. The increased monocytes observed in heifers fed LY also suggests that LY increased the innate immune response; which would allow for increased phagocytic activity within the infected area.

The acute phase response is induced by pro-inflammatory cytokines, which are protein hormones that act as messengers between local site of injury and hepatocytes which synthesize the acute phase proteins (Petersen et al., 2004). Haptoglobin is a positive acute phase protein that binds free hemoglobin in blood circulation and creates a haptoglobin-hemoglobin complex, which is thought to sequester and limit the amount of Fe available for bacterial proliferation (Richeson et al., 2016; Petersen et al., 2004). Heifers in the VB challenge exhibited a rapid increase in haptoglobin following the MH inoculation. The increase in haptoglobin due to MH inoculation is often reported and repeatable (Corrigan et al., 2007; Theurer et al., 2013; Burciaga-Robles et al., 2010b). This increase in haptoglobin suggests the animal's immune system stimulated an acute

phase protein response, and validates the challenge model. Interestingly, in the current study LY supplementation delayed the nadir of the haptoglobin by 1 d. Furthermore, heifers supplemented with LY had overall less haptoglobin produced than the controls. Similarly, Word et al. (2016) reported that supplementation with a combined live *Saccharomyces cerevisiae* and cell wall extract, tended to reduce serum haptoglobin concentration in heifers challenge with BHV-1 and MH. Results from these studies suggest the LY supplementation can reduce the inflammatory response, and reduce the catabolic effects associated with the acute phase response.

Total leukocytes were increased the day following inoculation with MH, which is in agreement with previous studies (Corrigan et al., 2007; Hanzlicek et al., 2010; Burciaga-Robles et al., 2010; Gånheim et al., 2005). A slight neutrophil increase due to BHV-1 inoculation was measured on day 3. Gånheim et al. (2005) reported no changes in circulating neutrophils due to BVD challenge, and Burciaga-Robles et al. (2010b) presented a reduction in neutrophils due to BVD exposure. The impact that viral challenges have on neutrophil production is unclear. However, neutrophilia in response to MH challenge is repeatable and indicates challenge success (Corrigan et al., 2007; Hanzlicek et al., 2010; Burciaga-Robles et al., 2010b; Gånheim et al., 2005). Heifers supplemented with LY had greater overall circulating neutrophils compared to the controls. Contrary to these findings Word et al. (2016) reported that supplementation with *Saccharomyces cerevisiae* tended to reduce neutrophils relative to controls in a combined BHV-1 + MH challenge. Differences between these results could be situational, or due to variation between the strains of live yeast. In the current study, the

sub-species *boulardii* was supplemented, which behaves different than *cerevisiae* (Fietto et al., 2004). Although, there was no effect of day, supplementation with LY may improve the efficacy of the innate immune response through increased neutrophil concentration.

Transient lymphopenia in ruminants is commonly caused by the acute phase of systemic infectious diseases (Smith, 2015). In the current study, the VB challenge decreased circulating lymphocyte concentrations. Similarly, Burciaga-Robles et al. (2010b) and Gånheim et al. (2005) reported decreases in lymphocytes due to MH and BVD challenges. However, Corrigan et al. (2007) and Hanzlicek et al. (2010) reported no changes in circulating lymphocytes following MH challenge. The cause of the inconsistency between the studies is unknown; however, the reductions in lymphocytes in the current study further validate the challenge model's ability to illicit an immune response.

The reductions in erythrocytes and hemoglobin from the VB challenge are most likely associated with inflammation of lung tissue. The mild anemia resulting from MH challenge has been reported previously (Corrigan et al., 2007; Hanzlicek et al., 2010; Burciaga-Robles et al., 2010b). Hematocrit is calculated as the proportion of erythrocytes to MCV. There were no differences in MCV throughout the study, and the differences in hematocrit are likely due to reductions in erythrocytes. Similarly, MCHC is computed as the proportion of hemoglobin to hematocrit and the observed differences are a result of decreased erythrocyte and hemoglobin concentrations.

Conclusion

The VB challenge model successfully stimulated an innate immune response which resulted in rhinitis with lesions, hyperthermia, increases in circulating haptoglobin and alterations to the hemogram synonymous with an acute infection. Furthermore, the VB challenge reduced DMI and suppressed ADG. Results from this study highlight the value of continuous remote monitoring of RUT over rectal temperature, due to the increased accuracy of RUT. Supplementation with LY increased neutrophils and monocytes, the two leukocytes most associated with an innate immune response. Furthermore, LY supplementation reduced haptoglobin concentration response in VB-challenged heifers, which indicates a reduction in the acute phase protein response. Despite the mitigation in haptoglobin concentration due to LY supplementation, the VB challenge induced reduction in DMI and performance was not affected by dietary treatment in this study. These results suggest that LY supplementation may be beneficial at reducing the catabolic effects associated with the acute phase response while increasing functionality of the innate immune system.

CHAPTER 5

EVALUATION OF STATISTICAL PROCESS CONTROL PROCEDURES TO MONITOR FEEDING BEHAVIOR PATTERNS AND DETECT ONSET OF A NATURALLY OCCURING BOVINE RESPIRATORY DISEASE OUTBREAK IN GROWING BULLS

Introduction

The objective of this study was to evaluate the effectiveness and accuracy of monitoring feeding behavior patterns using cumulative summation (CUSUM) procedures to predict the onset of bovine respiratory disease (BRD) in beef cattle. Growing bulls (N = 231) consigned from independent producers for the purpose of evaluating growth efficiency during a 70-d trial were used in this study. Within a 10-d period, 30 bulls were treated for BRD based on observed clinical symptoms and elevated rectal temperature (> 39.5 C); all remaining bulls (n = 201) were deemed to be healthy. The clinically ill and healthy bulls were used to evaluate the sensitivity and specificity of CUSUM models, respectively. Frequency and duration of bunk visit (BV) events, head down (HD) duration, DMI, eating rate, maximal non-feeding interval (NFI Max), SD of non-feeding interval (NFI SD) and time to bunk (TTB) were continuously monitored on a daily basis during this study. All data were standardized prior to generating CUSUM charts in a daily accumulative manner, which were constructed with PROC CUSUM (SAS 9.4). Accuracies of these univariate CUSUM models for detection of BRD were 69.4, 72.4, 79.1, 80.1, 63.7, 64.6, 73.2 and 48.7% respectively, and average day of

detection prior to observed symptoms of BRD were 3.2, 3.2, 4.8, 1.0, 10.2, 2.7, 1.5 and 0.6 d, respectively. In addition, principal components analysis (PCA) of all 8 univariate traits (full model) were used to construct multivariate factors that were monitored with CUSUM as previously described. Two reduced multivariate models were also constructed that included the 3 best performing feeding behavior traits (BV duration, HD duration, NFI SD) with (RBD) and without DMI (RB). The accuracy of the full model was similar to the univariate traits (75.0 %), the reduced models out performed all of the univariate traits and were equally accurate (84.0 %). All of the PCA models signaled prior to clinical observation ($P < 0.05$); the days prior for the full model, RBD model and RB model respectively were 2.1, 2.1 and 2.0 -d. These results demonstrate that the use of PCA-derived multivariate variables in CUSUM charts was more accurate compared to univariate CUSUM charts. Furthermore, the accuracy of the RB multivariate model was numerically better than the univariate models and adding DMI to the RB model did not further improve the accuracy or signal day. Moreover, due to the multivariate aspect of PCA, the use of PCA-based CUSUM charts to monitor feeding behavior patterns should be more robust in applications for preclinical detection of BRD. Results from this study demonstrate the potential value of using CUSUM procedures to monitor electronic feeding-behavior systems to enable accurate preclinical detection of BRD in feedlot cattle.

Mortality and morbidity challenges associated with bovine respiratory disease complex (BRD) continue to negatively impact the feedyard sector of the beef industry from both economic and animal welfare perspectives (Galyean et al., 1999; Duff and

Galyean 2007; Schneider et al., 2009). Respiratory disease accounts for 67 to 87% of the morbidity events within feedyards (Edwards, 1996), which is exacerbated when animals are stressed due to weaning, heat, shipping or co-mingling (Schneider et al., 2009). Early detection of BRD is difficult and current industry approaches rely on visual appraisal of clinical symptoms (Broom, 2006), which are not always accurate. Estimated sensitivity and specificity values for detection of BRD based on visual observations of clinical symptoms are highly specific but not very sensitive (92 and 27%, respectively, Timsit et al., 2016). The limitation of relying on clinical observations for disease detection is that prey animals have developed inherent instincts to conceal clinical symptoms of illness as an evolutionary adaption for survival (Noffsinger and Locatelli, 2004).

Recent developments in sensor technologies have enabled real-time measurements of behavior patterns (Theurer et al., 2013) and feed intake on an individual animal basis (Kayser and Hill, 2013; Lancaster et al., 2009), which have been shown to be predictive of morbidity events in beef cattle (Quimby et al., 2001; Sowell et al., 1999). These data collection systems coupled with robust mathematical models have utility in the development of an animal-health monitoring system for more accurate preclinical detection of BRD. Improvement in the sensitivity and specificity of preclinical disease detection would improve the efficacy of the antimicrobial treatment (Cusack et al., 2003) and lead to more judicious administration of antimicrobial therapy (Schafer et al., 2007).

Statistical process control (SPC) charts were first proposed by Shewhart (1931) to monitor abnormal variance of a process and were first applied in the manufacturing

industries, but their applications have also been used in the service, financial and health care industries (De Vries and Reneau, 2010; Montgomery, 2009). To date, there has been limited use of SPC procedures within the livestock industries. The objectives of this study were to evaluate the effectiveness of monitoring feeding behavior patterns, DMI and multivariate traits using cumulative summation (CUSUM; type of SPC) procedures to identify animal behavior deviations relative to the onset of disease in growing bulls undergoing a spontaneous outbreak of BRD.

Materials and Methods

Animals and Experimental Design

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee. Growing purebred bulls (N = 231) consigned from independent producers for the purpose of evaluating performance and feeding efficiency were used in this study. Although all bulls were previously vaccinated against viral and bacterial pathogens using various vaccine products, all bulls were re-vaccinated upon arrival at the test facility for bovine herpes virus, parainfluenza-3 virus, bovine viral diarrhea, bovine respiratory syncytial virus (Pyramid 5, Boehringer Ingelheim), and *Haemophilus somnus*, *Pasteurella multocida*, and clostridial diseases (Ultrabac7, Zoetis Animal Health), and treated for internal parasites (Valbazen, Zoetis Animal Health). Bulls were fitted with passive electronic identification (EID) ear tags (Half duplex; Allflex USA Inc., Dallas, TX), and adapted to the test diet for 28 d prior to

the start of a 70-d trial. During the trial, bulls were evaluated at least twice daily for clinical signs of illness and weighed at 14-d intervals.

Experimental Cohorts

Within a 10 d period beginning on day 28 of the trial, 30 bulls were identified as being morbid due to clinical observations of nasal discharge, lethargy and/or anorexia. Based on elevated (> 39.5°C) rectal temperatures (mean = 40.5°C [SD 0.05], range 39.7 to 42.1°C) during a physical examination, these bulls were diagnosed with BRD, treated with an antimicrobial (enroflaxacin; Baytril 100; Bayer Animal Health LLC., Shawnee Mission, KS) and returned to their respective home pens. These clinically-ill bulls were used to assess the true positive or sensitivity rate in evaluating the effectiveness of the CUSUM procedures. The remaining bulls (n = 201) were considered healthy and their feeding behavior responses were monitored to assess the true negative rate or specificity of the CUSUM chart. Although diagnostic tests were not conducted to confirm the presence of pathogens associated with BRD in this study, the observed clinical symptoms combined with elevated rectal temperatures in the clinically-ill cohort, were indicative of an acute outbreak of respiratory disease in these bulls.

Data Collection System and Behaviors

The bulls were housed in 1 of 9 pens (18.3 x 36.6 m) each equipped with 4 electronic feed bunks (GrowSafe System Ltd., Airdrie, Alberta, Canada) to measure daily DMI and feeding behavior traits. The GrowSafe system consisted of feed bunks

equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of EID tags. Assigned feed disappearance (AFD) rates were computed daily for each feed bunk to assess data quality. During the first 50 d of the feed intake data collected for this study, the mean AFD rates exceeded 99%. Therefore, no data were deleted due to system malfunction, power outage, or low AFD rates. Feeding behavior traits evaluated in this study were based on frequency and duration of bunk visit (BV) events, duration of non-feeding intervals (NFI), head-down (HD) duration and time to bunk (TTB). A BV event commenced when the EID of an animal was first detected, and ended when the time between consecutive EID recordings exceeded 100 s, was detected at another feed bunk, or when the EID of another animal was detected at the same feed bunk (Mendes et al., 2011). Bunk visit frequency was defined as the number of independent events recorded regardless of whether or not feed was consumed, and BV duration as the sum of the lengths of all BV events recorded during a 24-h period. The interval lengths between BV events were defined as NFI. Maximal NFI was defined as the longest NFI, and NFI SD as the SD of all NFI within each day. HD duration was computed as the sum of the number of times the EID for an animal was detected each day multiplied by the scan rate of the GrowSafe® system (1.0 s). TTB was computed daily as the interval length between time of feed-truck delivery within pen and each animal's first BV event following feed delivery. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance during each BV event. A subroutine of the

GrowSafe® 6000E software (Process Feed Intakes) was used to compute daily feed intake. Eating rate was computed as the ratio of daily DMI to daily BV duration.

Description of CUSUM

A SPC chart is a graphic display of a process over a time period. Shewhart (1931) first proposed the use of control chart methodology to monitor the variance and mean of a process to identify when a system goes out of control to improve product quality. Control charts contain a centerline, which represents the mean or target value of the process while in control, and upper or lower control limits that are based on the variance of the process (Figure 13). The control limits are set by the architect of the chart based upon the behavior of the process, and the process is deemed out of control when plotted observations exceed the bounds of these control limits (Montgomery, 2009, Quimby et al., 2001). This study utilized a one-way charting strategy, in which the chart only signaled in one direction. The upper threshold was used to monitor NFI Max, NFI SD, TTB and eating rate, whereas all others traits were monitored with the lower threshold of one-way charts.

Generally speaking, there are two types of charting strategies for monitoring a process. Shewhart charts are designed to identify mean shifts greater than 3 σ, and have no memory, in that current observations are not affected by preceding observations. Exponentially weighted moving average (EWMA; Roberts, 1959) and CUSUM charts (Page, 1954) directly incorporate all of the information in the sequence of sample values by plotting the cumulative sums of the deviations of the sample values from a target

value (Montgomery, 2009). Ultimately CUSUM, and EWMA charts behave in a similar manner, although the settings used to tailor the control charts for a specific application differ slightly.

The performance of a CUSUM chart is predicated upon the design, which includes the selection of K and H values. The parameter and CUSUM equations used in this study are presented in Table 13. The H-parameter values ranging from 1 to 5 were evaluated, to identify the optimal H-value for use in this study. Based on this evaluation, the parameter settings for this analysis were set as follows K = 0.5 and H = 3.5. All CUSUM charts were generated using PROC CUSUM in SAS 9.4 (Cary, NC).

Post Hoc vs. Daily Accumulative Parameter Estimation

Traditional methods of parameter estimation for CUSUM construction are conducted in a post-hoc manner, meaning that parameter estimates are calculated after all of the data has been collected. It is difficult to assess the accuracy and validity of these models, because they are utilizing future information that is unavailable in real-time application. For this study, initial parameter estimation was accomplished using the first 4 d as a reference period to calculate baseline μ and σ for each animal. Thereafter, daily observations were used to re-compute parameter estimates in an accumulative manner (Mertens, et al., 2008) using all of the available data up to that time point in the data set. Therefore, every sequential time series parameter estimate includes more data than previous observations, which enables the chart to behave as it would in real-time application. This daily accumulative procedure allows for more rapid implementation of

the monitoring process due to the relatively short reference period required for initial parameter estimation. Furthermore, the accuracy of these parameter estimates increase each day the process is being monitored, which allows the model to provide information about the behavior of individual animals shortly after feedlot arrival.

Standardization of the Observations

Transforming the observations to a standard normal basis has multiple advantages. In biological sciences, it is common to transform time-series observations to reduce the potential for autocorrelation. When standardized variables are used the procedure is known as standardized CUSUM, and is the primary methodology used to monitor biology (Mertens, et al., 2008; Quimby, et al., 2001; Montgomery, 2009). Furthermore, transforming all of the variables to the same scale minimizes the potential of over-weighting the importance of variables when included in principal components analysis (PCA), especially when the range in magnitude of variables is large and/or the unit of measurement differs across the variables (Johnson and Wichern, 2002).

For this study, data transformation was achieved using the following equation.

$$y_{ik} = \frac{(x_{ik} - \mu_{ij})}{\sigma_{ij}}$$

Where y_{ik} = the transformed daily observation for animal *i* on day *k*, x_{ik} = the daily observation for animal *i* on day *k*, μ_{ij} = the accumulative mean of the variable for animal *i* during time window *j* and σ_{ij} = the accumulative SD of the variable for animal *i* during time window *j*, and day *k* = maximal day during time window *j*.

Principal Components Analysis

Multivariate models, based on PCA analyses, were constructed using Proc Factor in SAS 9.4 (Cary, NC), with the eigenvalues calculated from the covariance matrix on an individual-animal basis. Dimension reduction was achieved by evaluating scree plots of the variance explained by the factors, ultimately resulting in the selection of 2 factors for each model. These 2 factors were monitored using the same CUSUM methods outlined for univariate traits. Based upon the performance of the individual factors, an "either or" rule was developed, such that if either of the factors signaled, the system was deemed "out of control". Conversely, all false positives were counted when either of the factors signaled "out of control" for the healthy cohort. Three multivariate models were evaluated including the full model that incorporated all 8 univariate traits, and a reduced feeding behavior model (RB) that included only the 3 highest performing traits (BV duration, HD duration and NFI SD) based on sensitivity and accuracy. The 3rd multivariate model (RBD) evaluated in this study included DMI along with the 3-traits within the RB model to assess the relative value of DMI to predict onset of BRD.

Model Accuracy Determinations

The clinically ill cohort was used to assess the sensitivity of the CUSUM procedure in monitoring the onset of BRD, as such all animals were considered to be morbid on the day they were treated. In order for the signal to be deemed a true positive (TP; signaled when the animal was morbid), the signal had to exceed the bounds of the

control limits and remain out of control up to 3 d prior to visual observation of illness. The healthy cohort was used to assess the specificity of the CUSUM procedure, this ensures that the chart does not signal when the animal is healthy. If the CUSUM statistic exceeded the H-value at any time the signal was classified as a false positive (FP; signaled the animal was sick when healthy). The inverse of these determinations were categorized as false negatives (FN; chart fails to signal when the animal is morbid) and true negatives (TN; fails to signal when the animal is considered healthy) respectfully. Ratios of theses classifications; sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy were used to evaluate the efficacy of the univariate and multivariate models for predicting onset of BRD (Figure 14). These diagnostic measures were calculated using PROC FREQ (SAS 9.4) for all univariate and multivariate models. Furthermore, 95% CI were computed within the FREQ procedure to identify statistical difference between the variables. This method would be synonymous to pair-wise T-tests except that the CI were computed using a chi-square distribution instead of a Gaussian distribution. A high performing model will exhibit high sensitivity, specificity, NPV, PPV, (close to 1.0) and therefore a high accuracy. The signal day is the average number of days prior to visual observation that the chart signaled the system was out of control. Proc Univariate (SAS 9.4) was used to construct 95% confidence intervals for signal day. Mean signal day for a trait was considered different from zero if the confidence interval did not span zero.

Results

H- Threshold

To determine the optimal H-parameter value for this study, CUSUM model sensitivity, specificity, and accuracy of several behavior response variables were evaluated at multiple H-parameter values ranging from 1 to 5 in 0.5 increments. Figure 15 graphically displays the relationship between sensitivity, specificity an accuracy for HD duration. As expected, the sensitivity decreased and the specificity increased as the H-parameter value increased from 1 to 5. The optimal H-parameter values based on sensitivity and specificity of the highest performing traits (BV duration, HD duration, NFI Max and DMI) ranged from 3.0 to 4.0. Thus, the H-parameter value of 3.5 was selected for use in this study.

Univariate Trait Models

The CUSUM model performance of the 6 feeding behavior and 2 DMI-based traits are presented in Table 14. The univariate trait with the greatest sensitivity was HD duration (76.7%), followed by BV duration and DMI that were equally sensitive (66.7%). These 3 traits were greater ($P < 0.05$) than TTB (23.3%), which had the lowest sensitivity, and HD duration was greater than NFI max (36.7%), which was the second least sensitive univariate trait. The remaining 3 traits (BV frequency, NFI SD and eating rate) had intermediate sensitivities that ranged from 46.7 – 53.3%, and did not differ from the other traits. There was greater segmentation of the univariate traits for specificity, which, is in part due to the degrees of freedom available for the CI

calculation. There were only 30 animals in the clinically ill cohort compared to 201 for the healthy group, therefore, there is greater confidence in the specificity estimates as well as narrower CI. Of the 8 univariate traits monitored DMI, NFI SD, NFI Max and BV frequency had greater ($P < 0.05$) specificity (92.0 – 93.5%) than eating rate, TTB, BV duration and HD duration (74.1 – 81.6%). Overall CUSUM performance assessed as accuracy was greatest for DMI (80.1%), but was closely followed by HD duration (79.1%). The feeding behavior traits NFI SD and BV duration were slightly less accurate than DMI, although still predictive of BRD, respectively their accuracies were 73.2 and 72.4%. The remaining univariate traits BV frequency, NFI max, eating rate and TTB were slightly less predictive, with respective accuracies of 69.4, 64.6, 63.7 and 48.7%.

Average signal day prior to observed clinical illness (Figure 16) were compared using 95% confidence intervals. Time to bunk was the only univariate trait with an average signal day that did not differ from 0. Eating rate signaled the earliest at -10 d, and was different ($P < 0.05$) from all other univariate traits. The average signal day for DMI and TTB (-1.0 and -0.6 d, respectively) were less ($P <0.05$) than the average signal day for HD duration (-4.8 d) and BV duration (-3.2 d), but did not differ from all other univariate traits. Although the average signal day for DMI and the other feeding behavior traits did not differ from each other, the average signal day for BV frequency and NFI max were numerically 2.2 and 1.7 d respectively, earlier compared to DMI.

Multivariate Trait Models

The model performance of the 3 multivariate models and their corresponding factors are presented in Table 15. Sensitivity, specificity and accuracy of the full model that used the "either or" rule were 70.0, 80.1 and 75.0%, respectively. Compared to the full model, both reduced models were more sensitive (83.3%), more specific (84.6%) and therefore were more accurate (84.0%). For all 3 of the multivariate models, the sensitivities and specificities for the 2 independent PCA factors were lower and higher, respectively, compared to the corresponding combined models that incorporated an "either or" rule of the 2 factors to detect when the system was out of control. As the magnitude of increase in sensitivity was greater than the reduction in specificity, the accuracies of the combined models exceeded that of the corresponding independent factors generated for the full and reduced multivariate models. The signal day for the 3 multivariate models (Table 15) were not statistically different from each other and ranged from -2.0 d for the RB model and -2.1 d for the RBD model.

Discussion

The objectives of this study were to evaluate the effectiveness of monitoring deviations in individual-animal feeding behavior traits and DMI using CUSUM procedures to predict the onset of BRD. Additionally, multivariate CUSUM models constructed using PCA were evaluated to determine if they were more sensitive and specific compared to the univariate traits.

Although there were no diagnostic tests performed to confirm the presence of specific pathogens associated with BRD in this study, the observed clinical symptoms combined with the elevated rectal temperatures were consistent with an acute outbreak of respiratory disease in these bulls. Furthermore, the results from Jackson et al. (2016) demonstrated that there was a marked reduction in DMI, as well as deviations in several feeding behavior traits in the clinically-ill compared to the healthy cohort, which is consistent with the onset of BRD.

There is widespread agreement that accurate preclinical-detection of BRD is crucial for effective intervention of this disease (Apley, 1997), which is the most prevalent and costly of disease complexes in beef cattle. The BRD complex accounts for the majority of morbidity challenges within feedyards (Edwards, 1996), and approximately 70% of the mortalities (Galyean et al., 1999). Identification of morbid cattle earlier in the disease process through objective behavioral monitoring would potentially improve the efficacy of antimicrobial therapy (Ferran et al., 2011), and therefore reduce mortalities as well as the duration of time animals are in a morbid state. The economic burden associated with BRD is more extensive than the direct losses associated with mortality; morbidity is the largest non-feeding cost associated with feeder cattle production (Pinchak et al., 2004), due to the increases in labor associated with treatment and production losses pre- and post-illness (Smith, 1998). When animals are morbid they are unproductive (Smith, 2015) because they divert energy from creating products (milk, muscle and fiber) to mount a defensive response to the disease. Many cases of BRD go untreated as diagnosis is difficult and relies on visual appraisal

of clinical illness (Broom, 2006). Numerous studies have documented poor to fair associations between observational detection of BRD and prevalence of lung lesions at harvest. In fact, White and Renter (2009) reported the sensitivity of BRD detection based on subjective observation of clinical signs was only 62%, which indicates that BRD cases often go undetected, or do not get detected until later in the disease process when successful intervention in less likely (Janzen et al., 1984). Moreover, the clinical symptoms of illness (e.g., lethargy, elevated temperature) typically associated with BRD are not exclusive for this disease, which limits specificity of BRD detection (63% White and Renter, 2009) resulting in overuse of antimicrobial drugs in feedlot cattle. Thus, there is a critical need to develop robust animal-health monitoring systems that are more sensitive in detecting BRD in order to improve efficacy of antimicrobial intervention and more specific to limit unnecessary use of antimicrobials, which would ultimately lead to improvement in animal welfare, profitability and perception of the industry.

Altered behavioral patterns associated with consumption of feed and water are among the earliest indicators of the onset of infectious disease. In calves at high risk for BRD upon feedlot arrival, Daniels et al. (2000) and Sowell et al. (1999) found that calves diagnosed and treated for BRD spent 23 to 42% less time at the feed bunk and had 10 to 36% fewer feeding and drinking bouts compared to untreated calves that did not display clinical symptoms of BRD. Frequency and duration of feeding bouts are known to be positively correlated with feed intake in beef cattle (Lancaster et al., 2009; Kayser et al., 2013). Thus, the calves that had BRD in these studies likely consumed less feed as evidenced by their lower daily gains. Carroll and Forsberg (2007) concluded that

the increase in energy required to produce pro-inflammatory cytokines, acute phase proteins, antibodies and mount febrile responses to infectious disease creates a state of hyper-metabolism. As such, animals compensate for this increased energy demand by altering various behavioral responses such as increased time for sleep, reductions in social activity, sexual behavior and feed intake in order to conserve energy. Jackson et al. (2016) used a 2-slope broken-line regression model to characterize deviations in DMI and feeding behavior patterns preceding the onset of observed clinical symptoms associated with BRD in cattle. The model-detected breakpoint for DMI occurred 6.8 d prior to observed clinical illness, whereas, breakpoints for BV frequency and duration were 7.6 and 7.2 d prior to observed clinical illness. Lukas et al. (2008) reported pre-clinical reductions in DMI in dairy cows with mastitis and reductions in water intake associated with the febrile response. Using pattern recognition techniques, Moya et al. (2015) reported that morbid cattle exhibit distinctive deviations in feeding behavior patterns prior to displaying overt clinical symptoms of BRD and can be differentiated from the feeding patterns of healthy cattle. Based on discrete survival time analysis of DMI and feeding behavior data, Wolfger et al., (2015) reported that increases in DMI per meal, meal frequency and inter-meal interval were associated with a decreased hazard for developing BRD up to 7 d prior to observed clinical symptoms of disease. The results from these studies suggest that deviations in feeding behavior patterns preceding the display of clinical symptoms of illness in beef cattle may be useful in development of predictive algorithms for preclinical detection of BRD.

Few studies have been conducted that have monitored changes in behavioral or physiological patterns of individual animals to predict the onset of disease. Other attempts at early identification have used hazard analyses (Wolfger et al., 2015), mean comparison (Sowell, et al., 1999), logistic regression (Schaefer et al., 2007) and cluster analyses (Moya et al., 2015). There are also studies and commercial products available that have published results without clearly defining the method or described the algorithm that was used to predict BRD. Using a novel high-frequency active integrated electronic system that measured BV frequency and duration MacGregor et al. (2015) monitored the health of high-risk calves and reported a reduction in morbidity and an increase in treatment success rate compared to visual observation of clinical illness. White et al. (2015), using the remote early disease identification (REDI) system were in agreement on morbid animals 94% of the time when compared to visual observation, and were able to identify morbidity 0.75 d prior to visual observation. The algorithms in the REDI system are proprietary, therefore they were not described, although it appears the predictions are made based upon a suite of behavior measurements.

Statistical process control procedures have been employed with success in multiple livestock species. Devries and Reneau (2010) reviewed 28 studies that employed SPC procedures to monitor various aspects of livestock production. Of their 28 studies, only 2 were conducted in beef cattle, with the remainder conducted in swine, poultry or dairy production systems. The traits that were monitored in these studies included morbidity, electro-conductivity of milk, muscle pH and conception rates. Furthermore, the majority of the studies were conducted on a herd or pen level rather

than on an individual-animal basis. Devries and Reneau (2010) concluded that carefully constructed control charts are powerful methods to monitor animal production systems, and that application of these methods will grow with advancement of biological sensor and computer technologies to monitor individual-animal health status and performance.

The H-parameter setting in SPC models is used to differentiate between abnormal and normal variation. The typical H-parameter value used in CUSUM models to monitor industrial processes is 5 (Montgomery, 2009). In this study, a H-parameter value of 3.5 was used, which was similar to the parameter setting used by Lukas et al. (2005) and Quimby et al. (2001) to monitor subclinical mastitis in dairy cattle and BRD in beef steers respectively. Not all univariate traits were most accurate at H = 3.5, that was the value that the high performing traits were on average the most accurate. The reduced H-threshold utilized in this study and others, suggest that the behavior changes are identifiable but exhibit less statistical distance from the mean than processes that are monitored outside of biology. These parameter differences illustrate the ability for the CUSUM procedures to accurately monitor processes with different underlying variation, and that not one set of parameters fits all systems. The parameters need to be tailored to the process being monitored.

Unique to this study was the accumulative methodology of parameter estimation, most CUSUM parameters (μ, σ) are estimated using post hoc methods (Mertens, et al., 2008), or the parameters are estimated once the study has been completed. The accumulative method does not compare an animal to it peers, and there is an inherent risk of flagging healthy outliers on the tails of animal populations when that method of

comparison is utilized. The CUSUM statistic calculated in this study is impacted by the daily fluctuations of the behavior an animal exhibits relative to the individuals' average/normal behavior.

Most of the studies that have monitored animal health have all used high-risk type cattle, Quimby et al., (2001) monitored cattle that had an overall pull rate of 67% compared to 13% in the current study. Other morbidity rates range from 77% (Wolfger et al., 2015) to 29 % (Moya et al., 2015), which are much higher than normal (mean morbidity rate = 14%, Irsik, et al., 2006). It is unknown whether using high-risk cattle to develop a predictive model would affect the ability of the model to select morbid animals in a normal population. Using high risk cattle certainly increases the probability of true positives.

Of the 6 feeding behavior traits evaluated in this study, HD duration, BV duration, and NFI SD had the highest sensitivity and accuracy. Furthermore, HD duration was similar in model accuracy with DMI. The only other study that monitored BV traits with a CUSUM procedure was Quimby et al. (2001) who reported greater accuracy and sensitivity of BV duration than the results of the current study. They were able to detect morbidity on average 4.1 d prior to clinical symptoms with an overall accuracy, PPV and sensitivity of 87, 91 and 90 % respectively, and results from their analysis are the most accurate to date. Despite their accuracy, it is difficult to draw conclusions from the comparison of the two studies, because the parameters were estimated differently. Quimby et al. (2001) monitored deviations in duration measured over 3-h and used the post-hoc mean and SD from the healthy population of calves to

parameterize their CUSUM chart. The results are certainly an improvement over visual observation, but would be difficult to reproduce in application. When a signal is obtained on a chart designed with parameters that are independent of the observations, it is impossible to know whether the signal is caused by a process change or by an incorrect parameter value (Quesenberry, 1997, De Vries and Reneau, 2010).

Unique to this study was the monitoring of eating rate, TTB and NFI max although they were not very good predictors of morbidity, inclusion in the report increases the body of knowledge in regards to the behavior changes an animal expresses due to onset of BRD. One of the many benefits of the CUSUM procedure is the constant monitoring and ability to detect small sustained mean shifts. In the current study, eating rate, NFI max, NFI SD and TTB all tended to increase prior to when the animal became morbid. The increase in eating rate was also reported by Jackson et al. (2016) who showed that the inflection of the slope change occurred 1.32 d prior to observed clinical illness, where upon the coefficient for the slope increased. They also reported similar increases in TTB with the slope change occurring 1.48 d prior to the onset of clinical disease. Eating rate signaled the earliest of all the traits, but was not very accurate compared to the other traits and TTB was the only trait monitored in this study whose signal day did not differ from zero. In the current study, increases in NFI max and NFI SD were observed prior to when the animal became morbid, which is in agreement with Jackson et al. (2016). Contrary to these results, Wolfger et al. (2015) reported a reduction in the hazard for BRD with a 1-h increase in mean time between meals or inter-meal interval. The differences between these studies reflect differences in the

feeding behavior traits being monitored or the methods being used to detect shifts in response variables prior to onset of disease. However, increases in NFI max and NFI SD would be consistent with observed behavior of morbid animals not feeding or feeding irregularly. In the current study, when animals were in a morbid state they took longer to respond to the first feeding, ate at a faster rate, spent more time not eating and had greater variation in the time between meals compared to when they were healthy.

To the authors knowledge this is the first study that has monitored principal component factors within a CUSUM to identify morbidity in beef cattle. Principal components analysis is among the most popular methods for extracting information from multivariate data (Bakshi, 1998). Principal components are a linear combination of variables that have been geometrically rotated to maximize the variability while removing the collinearity of the process variables (Montgomery, 2009). The intent of this analysis is to define the orthogonal directions that explain the variability in the data, in order to reduce the amount of original variables that need to be monitored. The general objectives of PCA is to reduce the data being monitored and assist in interpretation (Johnson and Wichern, 2002). The value of PCA in the deployment of monitoring procedures is three-fold; reduce the number of charts that need to be monitored (current study 8 vs. 1), monitor the change and interaction of multiple traits and minimize multicollinearity of traits through orthogonal transformation.

The *a priori* expectation for this modeling procedure was that the full model would outperform the reduced models, and that the interactions and changes of all traits relative to the onset of BRD would be collective and the chart would signal earlier and

with greater accuracy than the reduced models. Although there were no statistical differences between the full and reduced models with regards to sensitivity and specificity, for both measures the reduced models were greater. Therefore, the reduced models were more accurate. One of the assumptions of PCA is that large variances are important and represent the underlying structure of the process, and those structures with lower variance represent error or "noise" (Shlens, 2005). The full model contained 8 variables, 4 of those variables were not very accurate in monitoring BRD, evident in the univariate trait analysis. These traits had greater variance than the traits that signaled during a BRD event and reduced the sensitivity of the full model. The reduced models slightly outperformed the full model.

The reduced models had the greatest sensitivity compared to the full model and the univariate traits, but they also had slightly reduced specificity. There is a delicate balance that needs to be achieved amongst these 2 metrics to evaluate model performance. If specificity is too low users will abandon or lose faith in the procedure because the chart will signal that the animal is morbid when the animal is healthy (De Vries and Renau, 2010). If the sensitivity is too low there will be a similar fate for the charting procedure because sick animals will be visually present, but the chart will say they are healthy. There needs to be consideration placed on the value or cost of an incorrect decision compared to the cost of intervention. Caulcutt (1995), described this as the ratio of selling price to inspection cost, and compared two types of production processes to illustrate this point, the 'widget' process (low value items at a very fast rate) and the 'high value' process (high value items at a very slow rate). It is the opinion of

the authors that SPC application for monitoring health in beef cattle fits the second scenario, because the cost of health inspection is relatively low compared to the value of the animal. Therefore, more relative value should be given to sensitivity compared to specificity when constructing CUSUM charts as well as when deciding what behaviors should be monitored. Even though favoring sensitivity will decrease specificity the need for visual monitoring and or human intervention would still be substantially reduced compared to current practice of visual appraisal of health, which includes monitoring every animal every day. Therefore, benefits of monitoring process would not only include improved application of antimicrobial therapy, but could also potentially reduce labor associated with animal health activities, which account for little over 1/3 of the costs associated with operating a feedyard (Jensen and Mark, 2010).

Other benefits of using PCA methodology in SPC models is that additional sensor technologies can be easily incorporated when available. There has been an increased interest in the development of sensor technologies for the purpose of preclinical detection of disease in livestock. The feed intake measurement system used in the current study has been widely used in research and production facilities to measure feed intake and feeding behaviors, but the widespread use of this system in commercial cattle feeding systems is limited due to cost and labor associated with operation of the intake monitoring system (Lukas et al., 2008). Currently, there are multiple data collection systems commercially available that quantify animal behavior and physiology, and have been used to predict morbidity. These systems measures variables such as; time spent at the feed bunk and water source (Buhman et al., 2000), real-time positioning

through ultra-wide band tag transmitters (White, et al. 2015), three-dimensional accelerometers quantify animal activity (Bayne et al., 2016) and infrared thermography (Schaefer et al., 2007). The SPC methods that were examined in this study have the ability to be applied to any of these biosensor collection systems.

As data collection systems become more robust and less expensive, more behavior traits could potentially be monitored adding to the accuracy of the predictions. Certain behaviors have been shown to be good predictors of morbidity, for instance Lukas et al., (2008) concluded that water intake can serve as an alternative to DMI for monitoring the health of and estrus of Holstein cows. Deviations is drinking behavior patterns have also been shown to be effective predictors of diarrhea outbreaks in growing pigs and signal 1 d prior to the outbreak (Madsen and Kristensen, 2005). A monitoring program that included more variables could undoubtedly increase the robustness and accuracy of the prediction, which in turn should increase industry adoption.

There were no differences in sensitivity, specificity or signal day between the RBD and RB models, suggesting that deviations in DMI contributed little additional value to the PCA model that used only feeding-behavior traits. This is relevant as the collection of individual-animal DMI data is relatively more expensive compared to the collection of behavioral traits. Both of the reduced models outperformed all of the single trait models, illustrating the value of the PCA analysis compared to the single trait models. The reduced multivariate models were also more sensitive and specific than the estimations of visual appraisal (White and Renter, 2009).

Conclusion

These results demonstrate that the use of PCA derived factors in CUSUM charts was more accurate compared to univariate CUSUM charts and the removal of DMI from RBD model had no effect on accuracy and signal day prior to observed symptoms, demonstrating the validity of monitoring feeding behavior traits. Moreover, due to the multivariate aspect of PCA, the use of PCA based CUSUM charts to monitor feeding behavior patterns should be more robust in applications for preclinical detection of BRD. This illustrates the value of an electronic data collection system coupled with robust prediction procedures to the beef industry, through identification of morbid animals prior to overt clinical signs of disease. Future research needs to be conducted testing other behaviors that could improve the prediction and development of a system that is easily deployable within a feedyard at a reasonable cost as well as development of an economic model for selection of parameters so that producers can tailor the statistical process control methods for their operation.

CHAPTER 6

EFFICACY OF STATISTICAL PROCESS CONTROL PROCEDURES TO IDENTIFY DEVIATIONS IN CONTINUOUSLY MEASURED PHYSIOLOGIC AND BEHAVIORAL VARIABLES IN BEEF STEERS RESULTING FROM AN EXPERIMENTAL CHALLENGE WITH *Mannheimia haemolytica*

Introduction

The objective of this experiment was to determine if statistical process control (SPC) procedures coupled with remote collection of feeding behavior patterns, accelerometer-based behaviors, and rumen temperature can accurately differentiate between animals experimentally inoculated with *Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS). Thirty-six crossbred steers (BW = 352 ± 23 kg) seronegative for MH were randomly assigned to receive bronchoselective endoscopic inoculation with MH or PBS. Electronic feed bunks were used to measure DMI and feeding behavior traits, accelerometer neck collars were used to measure feeding- and activity-behavior traits, and ruminal thermo-boluses were used to measure core rumen temperature. Data were collected for 28 d prior to and following inoculation. Steers inoculated with MH exhibited elevated levels of neutrophils and rumen temperature ($P < 0.02$), indicating that the MH challenge effectively stimulated immunologic responses. However, only 9 of the MH steers exhibited increased serum haptoglobin concentrations indicative of an acute phase protein response and 1 of these animals displayed overt clinical signs of disease. Shewhart charts (SPC procedure) were used for this analysis,

and sensitivity was computed using all steers (n = 18) in the MH challenge with a subset that included only steers (n = 9) that exhibited a haptoglobin response. Specificity was calculated using all PBS steers in both analyses. In the analysis that included only MH-challenged haptoglobin responsive steers, DMI and bunk visit (BV) duration had the greatest accuracy (89%), with accuracies for head-down (HD) duration, BV frequency, time to bunk and eating rate being less (83, 69, 53 and 61%, respectively). In general the accelerometer-based behavior traits (ingestion, rumination, rest, standing) were highly specific ranging from 100% for rest, rumination and standing to 82% for ingestion. However, the sensitivity of these traits were low (0 to 50%), such that the accuracies were moderate compared to the feeding behavior and rumen temperature traits. To address the diurnal nature of rumen temperature, data were averaged over 6-h intervals, and quarterly temperature models were evaluated separately. Accuracy for the 4th quarter rumen temperature was more accurate (78%) than all other quarterly temperatures (1st = 56%, 2nd = 50% and 3rd = 67%). These results indicate that Shewhart procedures can effectively identify deviations in feeding behavior and rumen temperature patterns for the purpose of sub-clinical BRD detection.

The bovine respiratory disease (BRD) complex represents the largest economic loss due to mortality and morbidity within the feedyard sector of the beef industry (Duff and Galyean, 2007; Schneider et al., 2009). Despite improvements in vaccines, antimicrobials and animal management strategies designed to prevent BRD, mortality related to BRD has tended to increase (Engler et al., 2014). Current BRD detection methods rely on visual observation, and are highly specific but lack sensitivity (92 and

27% respectively; Timsit et al., 2016), indicating that a large proportion of cattle develop BRD during the feeding period, but are never diagnosed or treated. Utilization of clinical observations for disease detection is incongruent with evolution, as cattle are prey animals that have developed instincts to conceal symptoms of illness from predators (Noffsinger and Locatelli, 2004).

Recent developments in sensor technologies enable real-time measurements of behavior patterns, ruminal temperature, feed intake and feeding behavior on an individual-animal basis (Theurer et al., 2013; Timsit et al., 2011; Kayser and Hill, 2013; Lancaster et al., 2009). These data collection systems, coupled with robust mathematical models, can accurately identify deviations in animal behavior prior to the onset of BRD. Increased accuracy of BRD detection would likely improve the efficacy of antimicrobial treatment and reduce the duration of sub-clinical disease leading to an improvement in animal welfare (Cusack et al., 2003; Schaefer et al., 2007).

Statistical process control (SPC) was initially proposed by Shewhart (1931) to identify atypical variation and was initially applied within the manufacturing industries, but their applications have also been used in the financial and health care industries (De Vries and Reneau, 2010; Montgomery, 2009). Previous studies have evaluated the use of SPC procedures on monitoring feeding duration and ruminal temperature prior to the onset of BRD in high-risk and Holstein calves (Quimby et al., 2001; Timsit et al., 2011). However, these reports used visual observations to determine the health status of the animals, based on the aforementioned inaccuracy of visual observation it is difficult to ascertain the accuracy of the SPC procedures for detection of preclinical BRD. Our

objectives were to determine the sensitivity, specificity and accuracy of Shewhart control charts in determining if steers were experimentally inoculated with *Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS). Further, determine the accuracy of 3 data collections systems: feed intake and feeding behavior, rumen temperature and accelerometer based behavior system in discriminating between the MH and PBS treatments.

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC # 2015-0379) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2015-068).

Animals

A total of 36 Angus crossbred steers (initial BW = 386 ± 25 kg) originating from the McGregor and Beef Cattle Systems herds belonging to Texas A&M University were used in this study. All animals were considered clinically healthy based upon daily observations for 28 d prior to challenge and were seronegative for MH determined by paired serum samples collected 45 d apart. Furthermore, all animals were confirmed negative for persistently infected bovine viral diarrhea virus (BVDV), through collection of an ear notch prior to study commencement, which was analyzed with the BVD antigen-capture ELISA (BVD-Ag ELISA).

Experimental Design and Treatment Arrangements

This analysis was performed on data previously collected to evaluate the effects of live yeast (*Saccharomyces cerevisiae boulardii* strain I-1079 at 25 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition) supplementation on animal performance prior to and post MH challenge. Therefore, steers were stratified by herd origin, initial BW, MH titer dilution, exit velocity and pre-study ADG, then a random number generator was used to assign steers to 1 of 4 treatments (9 hd/treatment) arranged in a 2 x 2 factorial array. Supplementation with live yeast, however, did not impact the SPC procedures. Therefore, for this analysis only two treatment groups will be considered: steers inoculated with MH (n = 18) or phosphate buffer solution (PBS; n = 18).

The MH inoculum was prepared as described by Mosier et al. (1995). Briefly, MH serotype A1 was grown on trypticase soy agar containing 5% sheep blood for 18 h at 37 °C in 7% CO_2. Colonies were inoculated into brain-heart infusion broth and incubated for 16 to 18 h at 37 °C with aeration. The bacteria were then centrifuged at 3,000 × g for 15 min at 4 °C and washed twice with PBS. After the second wash, the bacteria were centrifuged as before and the pellet was re-suspended in PBS at a final concentration of 1.2 to 1.4 × 10^9 CFU/10-mL dose. After preparation, the inoculum was placed on ice in a dark cooler and transported to the site of inoculation (approximately 17 km).

Steers were fed for 28 d prior to inoculation on day 0, to collect baseline data. In order to avoid any chance of PBS animals receiving MH via contamination of the instruments used for inoculation, the PBS treatment group was inoculated prior to the MH treatment group. The inoculations were performed with an endoscope as described by Theurer et al. (2013). Steers were restrained in a standard hydraulic squeeze chute that allowed more specific restraint of the head. An endoscope 1 m in length was inserted into the ventral meatus of one nostril and passed into the trachea to the level of the right apical lung lobe bronchi allowing visualization of the opening. A sterile bronchoalveolar lavage tube was inserted into the endoscope portal and passed until the tip of the lavage tube was visible emerging from the endoscope. At this point, the lavage tube was advanced another 1 to 2 cm into the opening of the right apical lung lobe bronchi. Once in place, steers in the PBS treatment group were administered 10 mL of PBS followed by a 60 mL flush of PBS for a total of 70 mL. Following treatment of all the PBS animals, the endoscope was disinfected with chlorhexidine solution and rinsed with saline. Subsequently, steers in the MH treatment groups were challenged with 10 mL of MH serotype A1 at 1.2 to 1.4 × 10^9 CFU/mL followed by 60 mL of PBS for a total of 70 mL. Following this procedure, steers in both treatment groups were observed for adverse effects of the challenge procedure.

Throughout the 56-d study all animals were group housed in 4 pens at Texas A&M University's Beef Cattle Systems Research Center in College Station, TX. Steers challenged with MH and PBS were comingled with equal number of each treatment in a

pen. Steers were offered a growing diet *ad libitum*, which was provided twice daily at 0700 and 1600 h.

Data Collection

Clinical Illness Scoring. All steers were monitored by two experienced evaluators twice daily for clinical signs consistent with BRD. The visual evaluation employed in the experiment has been described in detail by Step et al. (2008). The criteria included signs of depression, inappetence and respiratory distress. Evaluators assigned a severity score of 1 to 4, where 1 was assigned for mild, 2 for moderate, 3 for severe and 4 for moribund. Steers receiving a 3 or greater were removed from the pen and given a full medical evaluation. Rectal temperature was measured during the medical evaluation and if it exceeded 40.5 °C, antimicrobial therapy was administered. All steers were returned to their home pen after evaluation. Temperature readings, BW and administration of antimicrobial therapies were recorded for every animal that was examined for clinical signs consistent with BRD.

Hemogram. Blood samples (7 mL EDTA and 10 mL Vacutainer with no additive, Becton, Dickson and Company, Franklin Lakes, NJ) were collected via jugular venipuncture using an 18-gauge needle on days -4, 0, 1, 2, 3, 5, 7, 10 and 14, relative to challenge. The EDTA samples were immediately submitted to a commercial lab (Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX) for total and differential white blood cell determination. Blood counts were performed with an

automated hemocytometer (ADVIA 120, Siemens Healthcare Diagnostics, Tarrytown, NY) using the factory installed cattle setting (ADVIA 120 Multispecies System Software, Version 2.206 MS, Siemens Healthcare Diagnostics). The hemocytometer counts leukocytes, erythrocytes and platelets by optical scatter and fluorescence. Differential leukocyte percentages were determined by visual cell counts on modified blood smears and absolute counts were calculated using the total leukocyte count from the hemocytometer.

To harvest serum, samples were allowed to clot then were centrifuged at 3,000 x g for 20 min at 20°C, then stored in duplicate aliquots at -20°C until subsequent analysis. Serum haptoglobin concentration was determined at the West Texas A&M University Ruminant Health and Immunology Laboratory (Canyon, TX) with a commercial, bovine-specific sandwich ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR). The haptoglobin analysis had an intra- and interassay coefficient of variation of 11.4 and 16.1%, respectively. The haptoglobin measures were used as an indicator of the effectiveness of the challenge. Quantification of haptoglobin response was accomplished by calculating area under the curve (AUC) values for each steer in the MH treatment using Proc Expand in SAS 9.4 (SAS Institute, Cary, NC). Two separate analyses were performed: the first included all steers, the second included only steers from the MH whose haptoglobin AUC exceeded 20 mg/dL/d (Fig. 19).

Serum concentrations of cortisol were determined as described by Littlejohn et al. (2016) and Burdick et al. (2009). A solid phase radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX) using antiserum-coated tubes were prepared

according to the manufacturer's directions. Serum cortisol samples were analyzed in duplicate and concentrations were determined based on a standard curve generated from known concentrations of cortisol using Assay Zap software (Biosoft, Cambridge, UK). The minimum detectable cortisol concentration for this assay was 1.2 ng/mL, and the interassay coefficient of variation was 8.8 %.

DMI and Feeding Behavior. All pens were equipped with electronic feedbunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake and feeding behavior data on an individual-animal basis. The GrowSafe system consisted of feed bunks equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of EID tags. Assigned feed disappearance rates were computed daily for each feed bunk to assess data quality and averaged 98% throughout the 56-d study. Feeding behavior traits were based on frequency and duration of bunk visit (BV) events. A BV event commenced when the EID of an animal was first detected, and ended when the time between consecutive EID recordings exceeded 100 s, when the same animal was detected at another feed bunk, or when the EID of another animal was detected at the same feed bunk (Mendes et al., 2011). Bunk visit frequency was defined as the number of independent events recorded regardless of feed consumed, and BV duration as the sum of the lengths of all BV events recorded during a 24-h period. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance during each BV event. Head down (HD) duration was computed as the sum of the number of times

the EID for an animal was detected each day multiplied by the scan rate of the GrowSafe system (1.0 s). Time to bunk (TTB) was computed daily as the interval length between time of feed-truck delivery within pen and each animal's first BV event following feed delivery. A subroutine of the GrowSafe 6000E software (Process Feed Intakes) was used to compute daily feed intake. For this study, eating rate was computed as the ratio of daily DMI to daily BV duration.

Rumen Temperature. Radiofrequency biothermal boluses (ThermoBolus, Medria, Châteauborg, France) were inserted into the rumen of all steers prior to initiation of the study. The ThermoBolus continuously recorded reticulo-rumen temperature (RUT) at 5-min intervals. A proprietary algorithm was used to remove variation in RUT due to drinking events. To reduce the variance induced by the diurnal pattern of rumen temperature, summary statistics were computed for 6-h time periods, with: quarter 1 ranging between 0000 – 0600, quarter 2 ranging between 0600 – 1200, quarter 3 ranging between 1200 – 1800 and quarter 4 ranging between 1800 – 2400. Summary statistics were computed daily for the 4 quarters.

Accelerometer-Based Traits. The Feed Phone system (Medria, Châteauborg, France) is composed of the Axel collar and Radio Base station. Feeding and activity behavior traits are generated from data recorded by the Axel sensor that is placed on a collar and securely fitted around the steers' neck. The sensor consists of a micro-electromechanical tri-axial accelerometer that quantifies changes of inclination, lateral and vertical

accelerations on a continuous basis. Nine metrics are recorded over 5-min intervals, and is automatically transmitted to the radio base station and thereafter to a web-based data center. Processing algorithms on servers convert the raw data into animal behaviors and the most dominant behavior within a 5-min interval is reported (Delagarde and Lemonnier, 2015). There are 5 reported behaviors that are mutually exclusive; ingestion (feeding duration), rumination, rest, other activity (duration of unidentified behaviors) and over activity (estrus behavior) as well as standing which is not mutually exclusive with the other behaviors.

Statistical Process Control Procedures

Charting Procedure. A SPC chart is a graphic display of a process over a given time period. Shewhart (1931) first proposed the use of control chart methodology to monitor the variance and mean of a process to identify when a system goes out of control to improve product quality. Control charts contain a centerline, which represents the mean or target value of the process while in control, and upper or lower control limits that are based on the variance of the process (Fig. 17). The control limits are set by the architect of the chart based upon the behavior of the process, and the process is deemed out of control when plotted observations exceed the bounds of these control limits (Montgomery, 2009, Quimby et al., 2001).

This analysis utilized the Shewhart method with 3 signaling strategies; effect can signal in both directions, effect can only signal on lower threshold and effect can only signal upper threshold. Eating rate, rest, standing, over activity and other activity, were

all set to signal in either direction. The feeding behavior traits; DMI, BV frequency, BV duration and HD duration were set to only signal on the lower threshold. All of the rumen temperature measures were set to only signal on the upper threshold.

The performance of a Shewhart chart is predicated upon the design, which includes the selection of the σ threshold values. The σ threshold values were evaluated from 1 through 7 at intervals of 0.5 (Fig. 18). This was done to identify the optimal σ threshold for each effect and are presented in Table 16. All Shewhart charts were generated using PROC Shewhart in SAS 9.4 (Cary, NC).

Accumulative Parameter Estimation. Traditional methods of parameter estimation have been done in a post-hoc manner, meaning that the parameters are calculated after all the data has been collected. Therefore, it is possible for a chart to signal based on parameters that are estimated with data collected in the future, and it is difficult to assess the validity and accuracy of the monitoring process using this methodology.

For this study, initial parameter estimation was accomplished using the first 4 d as a reference period to calculate baseline μ and σ for each animal. Thereafter, daily observations were used to re-compute parameters in an accumulative manner (Mertens, et al., 2008) using all of the available data to that time point in the data set. Therefore, every sequential time series measurement includes more data in estimating model parameters than the previous observations. These parameters were used to transform the daily observations to a standard normal basis. Plotted values are the daily observation subtracted from the accumulating μ and divided by the accumulating σ. Utilizing the

accumulating methodology enables the chart to behave as it would in real-time application. This procedure also allows for rapid implementation of the monitoring process due to the relatively short reference period required for initial parameter estimation. Furthermore, the accuracy of those parameters increases every day the process is monitored.

Model Accuracy Determinations. The MH challenge group was used to assess the sensitivity of the Shewhart procedure. In order for the signal to be deemed a true positive (TP, effect signaled for steer after inoculation) the signal had to exceed the bounds of the control limits. Steers challenged with PBS were used to assess the specificity of the Shewhart procedure to ensure the chart would not signal when an animal is healthy. If the effects value exceeded the threshold at any time the signal was classified as a false positive (FP, effect went out of control on a PBS steer). The inverse of these determinations were categorized as false negatives (FN, chart fails to signal when steer challenged with MH) and true negatives (TN, fails to signal in PBS challenge steers), respectfully. Ratios of these classifications: sensitivity, specificity and accuracy were used to evaluate the efficacy of the monitoring systems for identifying when an animal becomes morbid. These diagnostic measures were calculated using PROC FREQ (SAS 9.4). Furthermore, 95% CI were computed within the FREQ procedure to identify statistical difference between the variables, this method would be synonymous to pair-wise T-tests, except that the CI were computed using a chi-square distribution instead of a Gaussian distribution. A high-performing model will exhibit high sensitivity,

specificity and therefore a high accuracy. The signal day is the average number of days after inoculation that the chart signaled the system was out of control. Proc Univariate (SAS 9.4) was used to construct 95% CI for signal day.

Results and Discussion

Clinical illness scores were not different between the inoculation treatments (P = 0.65). Throughout the study, only one steer (MH treatment) received a clinical illness score ≥ 3 and during the health evaluation exhibited a rectal temperature > 40.5°C. The steer was treated with an antimicrobial, quickly recovered, and no re-treatments or mortalities occurred throughout the study. Gross clinical signs of disease resulting from the challenge model were not expected. Previous studies using a similar MH strain with intra-tracheal delivery, reported that challenged animals appeared clinically normal or slight increases in clinical illness scores that were not different from clinically normal (Capik et al., 2015; Corrigan et al., 2007).

The MH challenge in this study stimulated an immune response evident by increased ruminal temperature and circulating neutrophils. However, only half of the steers exhibited an increase (> 20, mg/dL/d) in serum haptoglobin concentration (Fig. 19). Characterizing the impact of the MH challenge on markers of inflammation is not the focus of this paper; however, the haptoglobin response appears to be indicative of the severity of the challenge and will lend clarity to the performance of the SPC procedures. Steers challenged with MH were categorized as haptoglobin responsive or non-responsive, based upon whether AUC of circulating haptoglobin concentrations

exceeded 20 mg/dL/d. This threshold was selected based upon plotted values and numerical separation between animals. Within the haptoglobin responsive group, the minimum haptoglobin concentration (33 mg/dL/d) response was 3-fold greater than the maximum haptoglobin concentration (11 mg/dL/d) response in the haptoglobin non-responsive group. There was no effect on the haptoglobin concentration response due to pen, LY supplementation or timing of inoculation.

The acute phase response is induced by pro-inflammatory cytokines, which are protein hormones that act as messengers between local site of injury and hepatocytes which synthesize the acute phase proteins (Petersen et al., 2004). Haptoglobin is a positive acute phase protein that binds free hemoglobin in blood circulation and creates haptoglobin-hemoglobin complexes, which is thought to sequester and limit the amount of Fe available for bacterial proliferation (Richeson et al., 2016; Petersen et al., 2004). In the current study, half of the steers challenged with MH exhibited an increase in haptoglobin concentration, which may be due to numerical increase of cortisol concentrations (Fig. 21). Although not statistically significant, non-responsive MH-challenged steers had numerically higher cortisol concentration then MH-challenged haptoglobin responsive steers the day following challenge. In support of these findings, Richeson et al. (2016) reported that haptoglobin concentration responses to a multi-valent respiratory vaccine were reduced/attenuated in animals that received prior injections of dexamethasone. The authors concluded that dexamethasone, which has been used for many decades as an anti-inflammatory, modulated the production of pro-

inflammatory cytokines, namely IL-6, which reduced production of acute phase proteins (Richeson, et al., 2016).

Haptoglobin responsive MH-challenged steers had greater ($P < 0.05$) circulating haptoglobin concentration than PBS-challenged and MH-challenged non-haptoglobin responsive steers from days 2 through 5 after challenge. There were no differences ($P > 0.05$) in haptoglobin concentration between the non-haptoglobin responsive MH-challenged and PBS-challenged steers for any day following inoculation. Steers challenged with MH exhibited greater ($P < 0.05$) ruminal temperature than PBS inoculated steers following inoculation. Furthermore, MH-challenged haptoglobin responsive steers had a greater ($P < 0.05$) rumen temperature than non-responsive MH-challenged steers. Similarly, all MH-challenged steers had increased ($P < 0.05$) concentration of circulating neutrophils compared to PBS-challenged steers following inoculation. As with rumen temperature, MH-challenged haptoglobin responsive steers had greater ($P < 0.05$) circulating neutrophil concentration 1 d after inoculation then MH-challenged non-haptoglobin responsive steers.

Steers challenged with MH were used to estimate the sensitivity of the SPC algorithms. Due to the aforementioned variation in rumen temperature, neutrophils, and haptoglobin within the MH challenge population, sensitivities of detection algorithms were evaluated on the complete MH population (Table 16) and a subset which calculated sensitivity with the haptoglobin responsive MH-challenged steers only (Table 17). Both analyses included the entire PBS population to estimate specificity. The results in Table 16 are included for completeness and to allow the reader to evaluate the results.

However, it would be redundant to discuss both tables; therefore only Table 17 will be discussed, where sensitivity was estimated using haptoglobin responsive MH-challenged steers (n = 9) only. Three different types of data collection systems are represented and evaluated for their effectiveness in combination with the SPC procedures for detecting BRD. Throughout this discussion it is relevant for the reader to consider the severity of the challenge, which was mild, and that these systems would potentially be more accurate when monitoring a naturally occurring BRD case.

Generally speaking, there are two types of charting strategies for monitoring a process. Shewhart charts are designed to identify mean shifts greater than 3 σ and have no memory, meaning current observations are not affected by preceding observations. Cumulative summation charts (Page, 1954) incorporate all information in the sequence of sample values by plotting the cumulative sums of the deviations of the sample values from a target value (Montgomery, 2009). Shewhart charts are more accurate at detecting large mean shifts, while cumulative summation charts detect small sustained mean shifts. Both cumulative summation and Shewhart charts were calculated; however, the physiological and behavioral responses due to MH challenge were more accurately detected with the Shewhart compared to the cumulative summation procedures. Thus, only the Shewhart procedure results are presented.

All SPC procedures require the design architect to set the thresholds for the process that will signal when the process has changed. The process threshold limits are designed to separate between normal and atypical variation. When the limits are low, the SPC procedure will have high sensitivity and low specificity. As the thresholds are

increased, sensitive decreases and specificity increases (Fig. 18). Results for the physiological and behavioral traits are presented at the σ threshold at which the response variable was most accurate (Table 16 and 17).

One of the most accurate feeding behaviors was DMI with a sensitivity of 78% and specificity of 100% (equal with BV duration, accuracy = 89%). Reductions in DMI are frequently observed in clinically ill cattle, and inappetence is often used within clinical illness scoring rubrics (Daniels et al., 2000; Sowell et al., 1999; Jackson et al., 2016). However, most commercial feeding operations aren't equipped to measure daily individual intake, and the current cost associated with feed intake systems would preclude them from wide-spread deployment in commercial feeding operations. Bunk visit and HD duration had equal sensitivity of 89%; although BV duration had greater specificity of 89% compared to HD duration which was 78%. Similar to these results, Quimby et al. (2001) monitored BV frequency of high-risk calves with a CUSUM chart for the 3 h post-feed delivery. They reported a sensitivity of 85% and specificity of 95% resulting in overall accuracy of 90%. Furthermore, the CUSUM chart detected morbidity 4.5 d prior to standard detection by feedyard personnel. Comparisons between this analysis and others with regards to signal day is difficult. Previous studies have compared a disease detection system against visual observation, and the disease detection system consistently identifies animals prior to observation; therefore a negative valuable is desirable. The current analysis measures signal day as average duration from inoculation to when the algorithm signals, and therefore, it is impossible for the signal day to be < 0. However, we can make comparisons between the magnitude of duration

across behaviors in this study. Of the 6 feeding behaviors evaluated; DMI, BV duration, BV frequency and HD duration, all signaled in less than 1 d (0.14 – 0.25 d). For a disease monitoring system to be successful it needs to have high sensitivity, specificity and accuracy, and alert in the beginning stages of the disease process so treatment is effective. The accuracy of BV duration and DMI were equal, and HD duration was the second most accurate feeding behavior. This suggests that monitoring attendance at the feed bunk could be as effective as monitoring DMI. Theoretically, BV duration would be a less expensive measurement than DMI because it would not require the use of scales. In a head to head comparison of pen riders and a system that monitored BV frequency and duration, MacGregor et al. (2015) reported that the technology system had reduced initial treatment (19.6 vs. 38.2%; $P < 0.01$), improved treatment success rate (81.5 vs. 67.6%; $P < 0.01$) and overall reduced medication costs ($34 vs. $40; $P < 0.04$). These results suggest that BV frequency is a valuable behavior for monitoring the onset of BRD with repeatable results.

Time to bunk had the greatest sensitivity (100%); although it had the lowest specificity, resulting in a low overall accuracy (53%). Steers were weighed on a weekly basis prior to inoculation, and were comfortable traveling through the processing facility. However, either the increased time required to pass the endoscope the day of challenge or the frequency of weighing post-inoculation disturbed the PBS steers' time to bunk, which resulted in the low specificity and may have overestimated sensitivity. Eating rate had relatively low sensitivity (33%); although the specificity was high at 89%. Interestingly, eating rate did not signal as out of control until 4 d after the challenge

suggesting that this behavior has a delayed response to the challenge. Jackson et al., (2016) reported deviations in eating rate 1 to 3 d prior to the onset of BRD in growing bulls utilizing a broken-slope linear regression model. Interestingly, they observed an increase in eating rate for bulls prior to becoming morbid, and suggested that the bulls were feeding at an increased rate in an effort to conserve energy. In the current analysis, there was no specific direction in which eating rate signaled, which is why the algorithm was allowed to signal on both the upper and lower thresholds. Estimates of sensitivity for eating rate in the current analysis are poor; however, future research endeavors should continue to evaluate the mechanism for the delayed impact and direction in which the behavior changes as an animal becomes morbid.

The Feed Phone system is an accelerometer-based behavior monitoring system that is primarily used in dairy ruminant production. The accelerometer is attached to a collar and can differentiate between feeding and ruminating durations (Delagarde and Lemonnier, 2015). Although, it is uncommon to use neck collars in beef cattle production, and the collars would need to be adjusted regularly on growing animals, the device is enticing because of potential reuse, spreading the cost across multiple turns of feedyard placements. Ingestion, which is a proxy for DMI, was the most sensitive (82%) of all behaviors collected by the accelerometer. Rumination had high specificity (100%), in fact, it never falsely signaled on a PBS steer; however, the sensitivity was low (25%). Rumination is paramount to maintaining a healthy environment for the rumen microbiome, and follows a circadian rhythm (Beauchemin, 1991). Van Hertm et al., (2013) showed that there were reductions in night time rumination duration immediately

prior to lameness detection in dairy cows. Rumination has also been used to detect the onset of calving (Büchel and Sundrum, 2014). However in the current analysis, rumination provided little value in detecting MH challenge. Rest and standing had equal specificities of 100%; however, their sensitivity was poor. In fact, the sensitivity for standing was 0%, which is why the signal day column is blank for that behavior (Table 17). Over activity and other activity had equal sensitivities of 25% and comparable specificities of 71 and 77%, respectively. This resulted in overall accuracy of 48 and 51% for over activity and other activity, respectively. Reduced activity is a common observation in cattle afflicted with lameness or diseases such as BRD (Richeson et al., 2018). Carroll and Forsberg (2007) proposed the reduction in activity is a compensatory response related to increase energy demands required to produce pro-inflammatory cytokines, acute phase proteins, antibodies and mount a febrile response associated with the hyper-metabolic state infectious diseases create. Pillen et al. (2016), utilized pedometers to determine behavior alterations of high-risk cattle that were clinically diagnosed with BRD. Calves that succumbed to BRD reduced their activity up to 6 d prior to visual detection and the mean separation was greatest the day before detection. Furthermore, they reported reductions in standing time, step count and lying bouts the day before clinical detection. The authors concluded that activity information provided by the accelerometers may assist in detection and management of sick cattle, and the objective measurement would complement current subjective evaluations (Pillen et al., 2016). The differing results in the described and current study may be a result of the analysis method; Pillen et al. (2016) used a post-hoc time-series model and compared

healthy to clinically-ill calves. Other reasons for differentiation may be the pedometer is more precise in collecting activity data than an accelerometer on a collar or a naturally occurring BRD event exerts greater influence on activity than the MH challenge.

In general, the feeding and activity behavior traits measured by the accelerometer-based system were highly specific but not sensitive; therefore, either the sensor does not measure the behaviors with enough precision to detect those behavior changes or the behavior was not affected by the challenge. DMI and BV duration were both effective measurements at detecting the challenge and did not falsely signal on the PBS animals; therefore, it is surprising that ingestion did not perform better.

Rectal temperature is regularly used as an aid in the diagnosis of BRD and often is the only objective measurement evaluated when deciding the current health status of an animal (Smith, 2015). One of the challenges with rectal temperature is it can be difficult to obtain because it requires moving an animal from the pen to a handling facility for restraint (Rose-Dye et al., 2010). Rectal temperature is often a point-in-time measurement and not always indicative of BRD. Capik et al. (2015) studied the transmission dynamics among beef calves experimentally challenged with MH and reported that clinical illness scores did not correlate with rectal temperature. Hanzlicek et al. (2010) measured rectal temperature 3 times daily on beef steers experimentally challenged with MH, and reported that the 3 measures were statistically different from each other, and was greatest (morning = 39.6, noon = 39.4 and evening = 40.2 °C) in the early evening. Furthermore, the rectal temperature always exceeded their upper reference (39.5 °C) limit, which was attributed to high environmental temperatures and

the restraint stress associated with the physical examination. One of the benefits of using rumen biothermal sensors to monitor body temperature is that it is a continuous measurement, which enables determination of core body temperature patterns and deviations of patterns on an individual basis. Sensitivity (78%) was equal for the average, minimum and maximum rumen temperature measured during the 4th quarter of the day. Subsequently, temperature measured in the 4th quarter had greater accuracy than the other 3 quarters accuracy for average, minimum and maximum rumen temperature were 78, 86 and 81%, respectively. Furthermore, all 4th quarter rumen temperature metrics signaled the day of inoculation. The other 3 quarters were less accurate, which may be due to an increase in ruminal temperature variance associated with the steers' feeding activities. Timsit et al. (2011) monitored reticulo-rumen temperature in an effort to examine the efficacy of this biosensor to detect the onset of BRD in young Holstein bulls after arrival to a feedyard. Reticulo-rumen hyperthermia exhibited a positive predictive value of 73%, and in the bulls that were correctly identified, the hyperthermia response was observed 1 to 3 d prior to clinical symptoms. In concordance with these studies, Schafer et al. (2007) monitored ocular surface temperature with an infra-red camera and reported a positive predictive value of 80%. Furthermore, animals were identified by the camera 4-6 d prior to the onset of clinical symptoms of BRD. The high sensitivity measured in the current study and previous reports confirm the value in monitoring core body temperature for the detection of BRD.

Conclusion

There are many benefits of utilizing remote continuously measured data to evaluate animal well-being. These include: collecting an unbiased measurement (prey animal not reacting to presence), the ability to evaluate the animal status over a longer duration of the day (pen riders have a finite period in which they can evaluate an animal), more targeted application of antimicrobials, and potential reductions in labor costs associated with treating morbid cattle. In the current analysis DMI and BV duration were the most accurate behaviors for detecting the effects of the MH challenge, and 4th quarter minimum rumen temperature was nominally lower. Future research is required to create sensors that can precisely measure behaviors that are indicative of disease. Furthermore, these sensors need to be cost-effective for producers to ensure a reasonable return on investment. Results from the current study will aid in sensor development, as well as prove the concept and lead to acceptance of new methodologies for detecting BRD.

CHAPTER 7

EFFICACY OF STATISTICAL PROCESS CONTROL PROCEDURES TO IDENTIFY DEVIATIONS IN CONTINUOUSLY MEASURED PHYSIOLOGIC AND BEHAVIORAL VARIABLES IN BEEF HEIFERS RESULTING FROM AN EXPERIMENTAL COMBINED VIRAL-BACTERIAL CHALLENGE

Introduction

The objective of this experiment was to determine if statistical process control (SPC) procedures coupled with remote collection of feeding behavior patterns, accelerometer-based behaviors, and rumen temperature can accurately differentiate between animals experimentally inoculated with a combined viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on d 0 followed by endobronchial inoculation with *Mannheimia haemolytica* (MH)) challenge or phosphate buffer solution (PBS). Thirty-eight crossbred heifers (BW = 230 ± 16.4 kg) were randomly assigned to treatments by initial weight, ADG, BHV-1 and MH serum titers. Electronic feed bunks were used to measure DMI and feeding behavior traits, accelerometer neck collars were used to measure animal activity, and thermo-boluses were used to measure rumen temperature over 5-min intervals. Heifers in the VB challenge exhibited decreased ($P < 0.01$) ADG and DMI, as well as increased ($P < 0.01$) neutrophils and rumen temperature synonymous with a naturally occurring bovine respiratory disease (BRD) infection. However, none of the VB challenged heifers displayed overt clinical signs of disease. Shewhart and cumulative summation (CUSUM) charts were used for this analysis. Sensitivity was

computed using heifers in the VB challenge (n = 19), and specificity was calculated using the PBS heifers (n = 19). To address the diurnal nature of rumen temperature, summary statistics were computed over 6-h intervals, and quarterly temperature models were evaluated separately. In the Shewhart analysis DMI had the greatest accuracy (95%) followed by rumen temperature (94%) measured during the 2nd and 3rd quarter. Rest was most accurate of the accelerometer-based traits (89%), and meal duration (87%) and bunk visit (BV) frequency (82%) were the most accurate of the feeding behavior measures in the Shewhart analysis. Signal day was the least for 3rd quarter rumen temperature (2.5 d) followed sequentially by BV frequency (2.8 d), meal duration (2.8 d), DMI (3.0 d) and rest (4.0 d). Generally the CUSUM analysis was less accurate than the Shewhart, however DMI and rumen temperature were still the most accurate variables at 79 and 80%, respectively. Meal duration (58%), BV frequency (71%) and rest (74%) were less accurate in the CUSUM compared to the Shewhart analysis; although, rumination (78 vs. 58%) and ingestion (75 vs. 62%) were more accurate. In the CUSUM analysis average day to signal was greater with DMI, RUT and meal duration being 4.4, 5.0 and 3.7 d, respectively. These results indicate that Shewhart and CUSUM procedures can effectively identify deviations in feeding behavior, activity and rumen temperature patterns for the purpose of sub-clinical BRD detection.

The current method of disease detection within US cattle feeding operations relies on the visual observation of pen riders (Portillo, 2014). Limitations to this method include training of personnel, subjectivity and brevity (Richeson et al., 2018). Diagnostic estimates for accuracy of the method reveal that visual observation is highly specific but

lacks sensitivity (92 and 27% respectively; Timsit et al., 2016), resulting in a large proportion of cattle during the feeding period developing BRD but remaining undiagnosed. Sub-clinical BRD cases have been shown to reduce gain, meat quality and profitability of cattle feeding operations (Griffin, 2014).

Recent technological advancements in remote data collection sensors have garnered great interest from both the cattle feeding and dairy industries (Rutten et al., 2013; Richeson et al., 2018). These data collection systems, coupled with robust mathematical models, will allow cattle producers to make real-time health evaluations on individual animals (Richeson et al., 2018). Improving detection methods would reduce sub-clinical infections and increase the efficacy of antimicrobial treatment by identifying disease before it has progressed to the point where it is obvious (Schaefer et al., 2007; Portillo, 2014).

Statistical process control (SPC) is a field of mathematics that focuses on identifying changes in a process over time, and was initially applied in the manufacturing industries, but application spread to the service, financial and health care industries (De Vries and Reneau, 2010; Montgomery, 2009). Previous reports have evaluated SPC procedures on monitoring feeding duration and ruminal temperature (Quimby et al., 2001; Timsit et al., 2011); however, these reports used clinical observations as their control. Our objectives were to determine the sensitivity, specificity and accuracy of Shewhart and cumulative summation (CUSUM) control charts in determining if heifers were inoculated with an experimental viral-bacterial (VB) challenge or phosphate buffer solution (PBS). Further, to determine the accuracy of 3

data collections systems: feed intake and feeding behavior, reticulo-rumen temperature and an accelerometer-based behavior collection system in discriminating between the VB and PBS treatments.

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC # 2015-0379) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2015-068).

Animals

A total of 38 Angus crossbred heifers (initial BW = 230 ± 16.4 kg) from the McGregor cattle herd belonging to Texas A&M University were used in this study. All animals were considered clinically healthy based on daily observations for 27 d prior to challenge and were seronegative for *Mannheimia haemolytica* (MH) determined by a serum sample collected prior to study enrollment. Serum titers were evaluated for exposure to bovine herpes viruse-1 (BHV-1), and heifers that fell within the middle of the distribution were selected for use in the study. Furthermore, all heifers were confirmed negative for persistently infected bovine viral diarrhea virus (BVD), through collection of an ear notch prior to the study, which was analyzed with the BVD antigen-capture ELISA (BVD-Ag ELISA).

Experimental Design and Treatment Arrangements

This analysis was performed on data previously collected to evaluate the effects of live yeast (*Saccharomyces cerevisiae boulardii* strain I-1079 at 62.5 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition) supplementation on animal performance prior to and post VB challenge. The VB challenge consisted of intranasal inoculation of BHV-1 on day 0 and endobronchial inoculation with MH on day 3. Heifers were randomly allocated to treatment groups by initial BW, MH titer dilution, BHV-1 titer dilution, exit velocity and pre-study ADG. Supplementation with live yeast did not impact the SPC procedures. Therefore, for this analysis only two treatment groups will be considered: heifers inoculated with VB challenge (n = 18) or phosphate buffer solution (PBS; n = 18).

Throughout the study all animals were group housed in 8 pens at Texas A&M University's McGregor Research Center in McGregor, TX. Heifers were segregated in pens by treatment arrangement and replicated twice. To ensure that PBS treatment heifers would not be contaminated with BHV-1, the inoculation treatments were penned on opposite sides of a barn with the BHV-1 treatment on the prevailing downwind side. Heifers were offered feed ab libitum, which was provided daily at 0700 h. The diet (DM basis) contained 36.5% dry rolled corn, 26% corn dried distillers grains, 30% chopped alfalfa hay, 5% molasses and 2.5% dry mineral.

Prior to day 0, the inoculum for the BHV-1 challenge was prepared. Briefly, the Cooper strain of BHV-1 was used in this study, and was originally obtained from the United States Department of Agriculture's (USDA), Center for Veterinary Biologics in

Ames, IA. The virus was propagated by inoculating 12, 850 cm^2 roller bottles of Madin Darby bovine kidney (MDBK) cells. Cells were grown in Eagles minimum essential medium (EMEM) with Earles basic salt and 10% fetal bovine serum (FBS). Growth medium was poured off and the BHV-1 inoculated at 10-1 dilution onto roller bottles and allowed to adsorb for 1 h at 36 ± 2 °C in 5 ± 1% CO_2. After which, EMEM with Earles salts and 2% FBS was added and roller bottles were returned to 36 ± 2 °C in 5 ± 1% CO_2 until cytopathic effect was observed to be approximately 90%. Virus was harvested by freeze/thaw, and the virus containing fluid was centrifuged at low speed (500 x g for 20 min) harvested and stored at -70 °C. Virus was distended in media to achieve the desired concentration of 1 x 10^8 plaque forming units (PFU) per mL.

The MH inoculum was prepared as described by Mosier et al. (1995). Briefly, MH serotype A1 was grown on trypticase soy agar containing 5% sheep blood for 18 h at 37 °C in 7% CO_2. Colonies were inoculated into brain-heart infusion broth and incubated for 16 to 18 h at 37 °C with aeration. The bacteria were then centrifuged at 3,000 × g for 15 min at 4 °C and washed with PBS twice. After the second wash, the bacteria were centrifuged as before, and the pellet was re-suspended in PBS at a final concentration of 5.4 × 10^{10} CFU/10-mL dose. After preparation, the inoculum was placed on ice in a dark cooler and transported to the site of inoculation (approximately 174 km).

On day 0, 1 mL of either BHV-1 at 1 x 108 PFU/mL or saline was aerosolized into each naris with a 3 mL syringe fitted with and intranasal mucosal atomization device (MAD Nasal; Teleflex, Morrisville, NC). Heifers in the PBS treatment were

inoculated prior to heifers in the VB treatment, and the chute and processing area were disinfected after BHV-1 inoculation. On day 3, all heifers were brought back to the process facility and endoscopically inoculated with MH or PBS. In order to avoid any chance of PBS animals receiving MH via contamination of the instruments used for inoculation, the PBS treatment group was inoculated prior to the MH treatment group. The inoculations were performed with an endoscope as described by Theurer et al. (2013). Heifers were captured in a standard squeeze chute and heads were restrained with a halter specifically designed for cattle. An endoscope 1 m in length was inserted into the ventral meatus of one nostril and passed into the trachea to the level of the right apical lung lobe bronchi allowing visualization of the opening. A sterile bronchoalveolar lavage tube was inserted into the endoscope portal and passed until the tip of the lavage tube was visible emerging from the endoscope. The lavage tube was advanced another 1 to 2 cm into the opening of the right apical lung lobe bronchi. Once the lavage tube was in place, heifers in the PBS treatment group were administered 10 mL of PBS followed by a 60 mL flush of PBS for a total of 70 mL. Following treatment of all the PBS animals, the endoscope was disinfected with chlorhexidine solution and rinsed with saline. Subsequently, heifers in the MH treatment groups were challenged with 10 mL of *M. haemolytica* serotype A1 at 5.4×10^{10} CFU/mL followed by 60 mL of PBS for a total of 70 mL. No adverse effects due to the inoculation procedure were observed for either treatment.

Data Collection

Clinical Illness Scoring. Heifers were monitored by two experienced evaluators twice daily for the duration of the experiment for clinical signs consistent with BRD. The visual evaluation employed in the experiment has been described in detail by Step et al. (2008). The criteria includes signs of depression, inappetence and respiratory distress. The evaluators assigned a severity score of 1 to 4, where 1 was assigned for mild, 2 for moderate, 3 for severe and 4 for moribund. Heifers receiving a 3 or greater were pulled from the pen and given a full medical evaluation. Rectal temperature was measured during the medical evaluation and if exceeded 40.5 °C anti-microbial therapy was administered. All heifers were returned to their home pen after the evaluation. Temperature readings, BW and treatments were recorded for every animal that was examined for clinical signs consistent with BRD.

DMI and Feeding Behavior. All pens were equipped with electronic feed bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake and feeding behavior data on an individual-animal basis. The GrowSafe system consisted of feed bunks equipped with load bars to measure feed disappearance, and an antenna located within each feed bunk to record animal presence via detection of electronic identification tags (EID; HDX high performance EID, Allflex USA Inc., Dallas, TX). Assigned feed disappearance rates were computed daily for each feed bunk to assess data quality and averaged 98% throughout the 57-d study. Feeding behavior traits were based on frequency and duration of bunk visit (BV) and meal events. A BV event

commenced when the EID of an animal was first detected, and ended when the time between consecutive EID recordings exceeded 100 s, when the same animal was detected at another feed bunk, or when the EID of another animal was detected at the same feed bunk (Mendes et al., 2011). Bunk visit frequency was defined as the number of independent events recorded regardless of feed consumed, and BV duration as the sum of the lengths of all BV events recorded during a 24 h period. Head down (HD) duration was computed as the sum of the number of times the EID for an animal was detected each day multiplied by the scan rate of the GrowSafe system (1.0 s). The interval lengths between BV events, when animals are not at the bunk were defined as non-feeding intervals. These non-feeding intervals were used to calculate meal criterion, which is used to aggregate BV events into meals. To solve for the individual meal criterions, non-feeding intervals were $\log_{10}$ transformed and plotted in a frequency distribution graph using the Meal Criterion Calculation software (MCC; http://nutrition-models.tamu.edu), which utilizes the R statistical software (ver. 2.13; R Foundation for Statistical Computing; http://r-project.org) and the mixdist R package. Within MCC, bimodal Gaussian-Weibull probability density functions were fitted with the transformed non-feeding intervals. For each animal the meal criterion was defined at the intersection of the 2 probability density functions (Bailey et al., 2012). Once meal criterion was defined for each animal, BV events were aggregated into daily meal frequencies and durations. Feed intake was allocated to individual animals based on continuous recordings of feed disappearance during each BV event. A subroutine of the GrowSafe 6000E software (Process Feed Intakes) was used to compute daily feed intake.

Rumen Temperature. Radiofrequency biothermal boluses (ThermoBolus, Medria, Châteauborg, France) were inserted into the rumen of all heifers prior to initiation of the study. The ThermoBolus continuously recorded reticulo-rumen temperature at 5-min intervals. A proprietary algorithm was used to remove variation in RUT due to drinking events. To reduce the variance induced by the diurnal pattern of rumen temperature, summary statistics were computed for 6-h time periods, with: quarter 1 ranging between 0000 – 0600, quarter 2 ranging between 0600 – 1200, quarter 3 ranging between 1200 – 1800 and quarter 4 ranging between 1800 – 2400. Summary statistics were computed daily for the 4 quarters.

Accelerometer-Based Traits. The Feed Phone system (Medria, Châteauborg, France) is composed of the Axel collar and radio base station. Feeding and activity behaviors are generated using the Axel sensor, which is placed on a collar and securely fitted around the heifer's neck. The sensor consists of a micro-electromechanical tri-axial accelerometer that measures and continuously analyzes the changes of inclination, lateral, and vertical accelerations. A set of 9 statistical data are recorded every 5 min, automatically transmitted to the radio base station, and thereafter to a web-based data center. In the data center, processing algorithms on servers convert the raw data into standardized data. The algorithms determine the most dominate activity over a 5 min duration (Delagarde and Lemonnier, 2015). There are 5 reported behaviors that are

mutually exclusive: ingestion, rumination, rest, other activity and over activity. Standing is also recorded, which is not mutually exclusive with the other behaviors.

Statistical Process Control Procedures

Charting Procedures. A SPC chart is a graphic display of a process over a given time period. Shewhart (1931) first proposed the use of control chart methodology to monitor the variance and mean of a process to improve quality of manufactured goods. Control charts contain a centerline, which represents the mean or target value of the process while in control, and upper or lower control limits that are based on the variance of the process. In general, there are two types of charting strategies for monitoring the variation of a process. Shewhart charts are designed to identify mean shifts greater than 3 σ, and have no memory, in that current observations are not affected by preceding observations. Cumulative summation (CUSUM) charts (Page, 1954) directly incorporate all of the information in the sequence of sample values by plotting the cumulative sums of the deviations of the sample values from a target value (Montgomery, 2009). Both Shewhart and CUSUM charts were used in this analysis.

The performance of a Shewhart chart is predicated upon the design, which includes the selection of the σ threshold values. The σ threshold values were evaluated from 2 - 5 at 0.5 intervals. This was done to identify the optimal σ threshold for each response variable and are presented in Table 18. This analysis utilized 3 signaling strategies; variable can signal in both directions, variable can only signal on lower threshold and variable can only signal upper threshold. Rest, standing, over and other

activity were able to signal on either threshold. The feeding behavior traits, DMI, ingestion and rumination, were only allowed to signal on the upper threshold. All of the rumen temperature measures, were set to signal on the upper threshold. All Shewhart charts were generated using PROC Shewhart in SAS 9.4 (Cary, NC).

Threshold values within CUSUM charts are called H values, and selection of the correct value is paramount to the accuracy of the monitoring process. The current analysis evaluated H values from 1 through 6 at 0.5 intervals. The construction of a CUSUM chart also requires selection of a K value, which is an offset value, and prevents high numbers of small fluctuations from accumulating and causing the chart to signal. The CUSUM charts were evaluated at the previously mentioned H values with K = 0.5 and K = 0.75. The signaling strategies outlined in the Shewhart construction were also used for the CUSUM charts. All CUSUM charts were generated using PROC CUSUM in SAS 9.4 (Cary, NC).

Accumulative Parameter Estimation. Traditional methods of parameter estimation have been done in a post-hoc manner, meaning that the parameters (μ and σ) are calculated after all data has been collected. Therefore, it is possible for a chart to signal based on parameters that are estimated with data collected in the future, and it is difficult to assess the validity and accuracy of the monitoring process using this methodology. This study evaluated parameter estimation using 5 different time windows. All days included all of the available data, and the 7, 10, 14 and 20 d windows included the number of preceding days in the estimation. This is visually presented in Figure 22.

These parameters were used to transform the daily observations to a standard normal basis. Plotted values are the daily observation subtracted from the accumulated μ and divided by the accumulated σ. Utilizing the time window methodology enables the chart to behave as it would in real-time application, and reduces the potential for false positives. This procedure also allows for rapid implementation of the monitoring process due to the relatively short reference period required for initial parameter estimation.

Model Accuracy Determinations. The VB treatment was used to assess the sensitivity of the SPC procedures. In order for the signal to be deemed a true positive (TP, effect signaled for heifer after inoculation), the signal had to exceed the bounds of the control limits. The PBS treatment was used to assess the specificity of the SPC procedures, which ensures the chart would not signal when an animal is healthy. If the variables value exceeded the threshold at any time, the signal was classified as a false positive (FP, variable went out of control on a PBS heifer). The inverse of these determinations were categorized as false negatives (FN, chart fails to signal on VB heifer) and true negatives (TN, fails to signal on PBS heifer), respectfully. Ratios of these classifications: sensitivity, specificity and accuracy were used to evaluate the efficacy of the SPC procedures. These diagnostic measures were calculated using PROC FREQ (SAS 9.4). Furthermore, 95% CI were computed within the FREQ procedure to identify statistical difference between the variables, this method would be synonymous to pair-wise T-tests, except that the CI were computed using a chi-square distribution instead of a Gaussian distribution. A high-performing model will exhibit high sensitivity, specificity and

therefore, a high accuracy. The signal day is the average number of days after inoculation that the chart signaled the variable was out of control. Proc Univariate (SAS 9.4) was used to construct 95% CI for signal day.

Results and Discussion

Heifers in the VB challenge exhibited physiological and behavioral changes associated with an acute BRD infection. The VB-challenged heifers had reduced ADG ($P < 0.01$) and DMI ($P < 0.01$), increased leukocytes ($P < 0.01$), haptoglobin concentration ($P < 0.01$) and rectal temperature ($P < 0.01$; data not presented) compared to the PBS-challenged heifers. Inoculation with BHV-1 created nasal lesions and erosions, classically observed with viral rhinitis. However, there were no differences in clinical illness scores between the inoculations. In fact, only two heifers (VB inoculation) received a clinical illness score ≥ 3, and upon a medical evaluation did not have a rectal temperature > 40.5. There were no naturally occurring cases of BRD, re-pulls due to challenge, or mortalities during the study.

The inability of subjective visual observation to detect BRD in cattle has been well documented. Gardner et al. (1999) evaluated the impact of respiratory disease on steer performance during 150-d finishing period. At harvest, the animals lungs were evaluated for evidence of BRD, and 37% of the steers never diagnosed during the feeding period exhibited respiratory tract lesions. Furthermore, in steers that were diagnosed and treated for BRD, 52% did not have any evidence of respiratory tract disease at harvest. The authors concluded that this discrepancy could be due to treatment

of sub-clinical infections, imprecise disease diagnosis, full recovery of BRD due to treatment, or the steers were suffering from a viral infection that never manifested necrotic lesions in the lung tissue (Gardner et al., 1999). Multiple studies have documented the incongruency between treatment of BRD during the finishing phase and prevalence of lesions at harvest (Wittum et al, 1996; Buhman et al., 2000; Thompson et al., 2006; Schneider et al., 2009; Tennant et al., 2014). In an effort to assess the diagnostic accuracy of clinical illness determination for BRD, Timsit et al. (2016) utilized results from the previously mentioned studies and others within a Bayesian meta-analysis. Predicted sensitivity and specificity estimates were 27 and 92%, respectively. Concluding that clinical illness determinations exhibited poor sensitivity and high specificity. One of the challenges with using subjective evaluation to determine health status of cattle is the predator-prey relationship between the species. Prey animals have developed evolutionary instincts to conceal illness from predators and may only display symptoms with severe infection (Noffsinger and Locatelli, 2004). Ultimately, cattle in the US feed yards frequently succumb to undiagnosed BRD. The results from the current study are in agreement with these conclusions. Heifers in the VB challenge exhibited physiological alterations associated with BRD; however, the animals never displayed clinical signs of disease such as depression or lethargy. The behavioral changes induced by the VB challenge were only observed in the remote continuously recorded data.

Sub-clinical or undiagnosed BRD during the finishing period has been shown to reduce ADG and quality grade resulting in overall decreased value of the carcass

(Gardner et al., 1999). Reducing the rate of sub-clinical BRD could improve the return on investment up to 10%, depending on the economic climate (Griffin, 2014). A proposed solution to reduce sub-clinical morbidity has been to utilize sensor technology to objectively and continuously collect animal behaviors which can then be evaluated with disease detection algorithms (Richeson et al., 2018). The current study evaluates the use of Shewhart and CUSUM methods for detecting when physiologic and behavior response variables change in response to a VB challenge. The authors also used different windows of time to evaluate these measures. Process control procedures are employed to monitor a process over time and detect when the process changes. In livestock production, there are natural changes in physiology and behavior as the animals mature. The purpose of the differing duration of time windows was to evaluate the impact that the parameter estimation would have on the SPC methods. For example, 1 record in the 7 d window comprises 14% of the information in estimating that parameter, while 1 record in the 20 d window only comprises 5%. Therefore, the values in the 7 d window will fluctuate more closely towards 0, and be more resilient to an outlier falsely signaling that the process has changed. This is graphically presented in Figure 23, which shows the value of the CUSUM statistic for DMI. The CUSUM statistic is much slower to return to normal in the all days window due to the influence that low intake days have on the CUSUM statistic; however, the 7 d window returns to normal more quickly because the low intake values comprise a larger proportion of the mean.

Generally speaking the Shewhart charts were more accurate using the all day window, while the CUSUM performed better with the shorter duration time windows.

This is due to the differences between the methods with CUSUM exhibiting greater accuracy in detecting small mean shifts; therefore a shorter duration time window will increase the specificity if the trait being measured naturally changes over time. These time windows were incorporated into the analysis to provide a solution for monitoring time-series information that is expected to change over time, and a method for deciphering between expected change within a variable, and change due to disease.

There are multiple commercial products capable of direct and indirect measures of individual animal feed intake (Richeson et al., 2018). In the Shewhart analysis, DMI was the most accurate trait for detecting the challenge at 94%, and signaled the day of MH inoculation (Table 18, Figure 24). Similarly, DMI was the 2nd most accurate trait in the CUSUM with an accuracy of 79%, and signaled 1.4 d after MH inoculation on average (Table 19, Figure 25). Reduced DMI is a common clinical sign associated with BRD and has been the focal measurement for many disease detection systems (Duff and Galyean, 2007; Richeson et al., 2018). Jackson et al. (2016) used a 2-slope broken-line regression model to characterize deviations in DMI preceding the onset of observed clinical symptoms associated with BRD in cattle. The model-detected breakpoint for DMI occurred 6.8 d prior to observed clinical illness. Similarly, Lukas et al. (2008) reported pre-clinical reductions in DMI in dairy cows with mastitis and reductions in water intake associated with the febrile response. Current results and previous reports have shown that monitoring DMI is effective at detecting disease in cattle. However, there has been little industry adoption of these systems for disease detection. Most available intake monitoring systems require compartmentalization of the feeding area,

which can be intimidating to cattle and require significant acclimation period (Richeson et al., 2018). Furthermore, the cost and management of the systems limit their practicality for use as a remote data collection system within a commercial feed yard setting (Richeson et al., 2018).

A solution to capture the value of DMI for disease monitoring without directly measuring it is to use indicator traits such as frequency and duration of feeding events. Feeding behaviors, such as BV frequency and duration, as well as meal frequency and duration have been shown to be moderately correlated with DMI (Kayser and Hill, 2013; Lancaster et al., 2009). Furthermore, these feeding behavior traits are impacted by the onset of BRD. Sowell et al. (1998) reported that healthy steers had 30% greater BV duration than morbid steers. Using pattern recognition techniques, Moya et al. (2015) reported that morbid cattle exhibit distinctive deviations in feeding behavior patterns prior to displaying overt clinical symptoms of BRD and can be differentiated from the feeding patterns of healthy cattle. MacGregor et al. (2015), monitored the BV frequency and duration of high risk calves, and reported a reduction in morbidity and an increase in treatment success rate compared to visual observation of clinical illness. In the current study, BV frequency and meal duration were both effective at detecting the disease challenge in the Shewhart analysis with accuracies of 82 and 87%, respectively. Furthermore, these behaviors average signal day were equal at 2.8 d. This was prior to inoculation with MH, suggesting that these behaviors are more sensitive to viral infection. Similarly, White et al. (2015), were able to identify morbidity 0.75 d prior to

visual observation using the remote early disease identification (REDI) system and were in agreement on morbid animals 94% of the time when compared to visual observation.

Accuracies for BV frequency and meal duration in the CUSUM were 71 and 58%, respectively, which was considerably less than when monitored with the Shewhart chart. Sensitivities for BV frequency were equal for both the CUSUM and Shewhart charts; however, the specificity was considerably lower in the CUSUM. Conversely, the sensitivity was considerably lower for meal duration in the CUSUM compared to the Shewhart. Quimby et al., (2001) monitored BV duration of high-risk calves for 3 h after initial feeding with a similar CUSUM, and reported that the model was 90% sensitive and 87% accurate. Furthermore, CUSUM was able to detect disease 4 d prior to visual observation. In the current study, the Shewhart method was more accurate at detecting the VB challenge than CUSUM for DMI and feeding behaviors. Differences in accuracy of the CUSUM between the current study and Quimby et al. (2001) could be explained by the differences in pathology between an experimental challenge and a naturally occurring BRD outbreak. The VB challenge was mild, in that there were no clinical signs of disease or mortalities; however, the concentration of viral and bacterial pathogens are applied in greater concentration than found naturally. It is conceivable that Shewhart procedures are better at measuring the quick, transient change caused by a challenge, while CUSUM better detects a naturally occurring disease outbreak, which may take longer to manifest into disease. In either case these results show that SPC procedures are accurate at detecting the deviations in DMI and feeding behaviors in response to experimental challenge and naturally occurring BRD cases. Furthermore,

feeding behaviors appear to be more sensitive to the challenge model than DMI by signaling earlier. Collection of feeding behavior data does not require the use of compartmentalized feed bunks which may reduce the cost of the measurement, and increase industry adoption of the technology.

Accelerometers are non-invasive sensors that can quantify multiple physical behaviors dependent on where they are placed on the animal. These technologies have been tested extensively in dairy production systems for detection of locomotion abnormalities, estrus and mastitis (Rutten at al., 2013). Accuracies of the CUSUM for ingestion and rumination were 75 and 78%, respectively. The ingestion measurement would be similar to the BV or meal duration measurements, in that it records the time the animal spends feeding. The accuracy for ingestion was less in the Shewhart and greater in the CUSUM when compared to meal duration. Rumination was highly specific (94%) in the CUSUM and moderately sensitive (61%). Rumination is paramount to maintaining a healthy environment for the rumen microbiome, and follows a circadian rhythm (Beauchemin, 1991). Van Hertem et al., (2013) showed that there were reductions in night time rumination duration immediately prior to lameness detection in dairy cows. Rumination has also been used to detect the onset of calving (Büchel and Sundrum, 2014). In the current analysis, rumination was the most accurate of the accelerometer based traits monitored by the CUSUM and reductions in rumination would likely be due to reductions in DMI.

Rest monitored with the Shewhart method was the most accurate of the accelerometer-based traits (89%), and signaled on average the day following (d 4)

following inoculation with MH. With the exception of standing, rest is mutually exclusive with the other traits; therefore, an increase in rest is due to a decrease in all other measures. Over activity, other activity and standing durations provided little value in either the Shewhart or CUSUM procedures. Standing or lying measured by accelerometers attached to hind limbs has been shown to be influenced by disease challenge models and naturally occurring BRD outbreaks. Hanzlicek et al. (2010) used an accelerometer/pedometer device to quantify changes in animal behavior due to an experimental challenge with MH. They concluded that the accelerometers were not useful in determining disease progression as measured by pulmonary lesions, but the sensors were useful at characterizing the behavior changes induced by the challenge. Prior to challenge the steers spent more time standing than lying; although 4 d after the challenge, steers spent more time lying. These results were similar to Theurer et al. (2013), who reported increases in lying time in heifers challenged with MH. Pillen et al (2016), utilized pedometers to identify behavior alterations in high-risk calves prior to a naturally occurring BRD outbreak. Morbid calves displayed reductions in activity up to 6 d prior to clinical signs of disease. Furthermore, morbid calves had decreased standing duration, lying bouts and total step count the day prior to detection. In the current study, standing duration provided little value for detecting the challenge. One of the differences between the current study and others could be that standing is more precisely measured on the hind limb rather than when the accelerometer is attached to a collar. However, rest in the current study was highly accurate in the Shewhart chart, and may be a better comparison to the lying duration reported in the other studies.

Fever is a common symptom associated with BRD and has many beneficial effects for fighting an infection (Smith, 2015). The rise in body temperature typically occurs within 24 h of initial infection; however, it is commonly unnoticed by animal health monitoring personnel (Voss et al., 2016). In fact, Timsit et al. (2011) reported that out of 449 rumen temperature fever episodes, only 108 (24%) were associated with visually detected signs of disease. Furthermore, the duration in which animals spent in an undiagnosed febrile state was associated with decreased ADG. Body temperature is typically measured rectally, which is labor intensive and often follows detection of visual signs of disease (Rose-Dye et al., 2011). Efforts to remotely quantify body temperature have included the use of infrared thermography, inner ear canal thermistors and thermal boluses (Sellier et al., 2014). In the current study, rumen temperature was highly accurate at detecting the VB challenge. The 2nd and 3rd quarter average rumen temperature monitored with the Shewhart chart had equal sensitivity and specificity of 89 and 100%, respectively. Furthermore, the 3rd quarter rumen temperature measurements signaled approximately 12 h prior to MH inoculation. The CUSUM procedures were less accurate at monitoring rumen temperature than the Shewhart due to decreased specificity of the CUSUM. Voss et al. (2016) monitored rumen temperature with a CUSUM to detect BRD in 17 d old Holstein-Friesian calves. They reported sensitivity and specificity values of 71 and 98%, respectively, and BRD was identified 3.5 d earlier than clinical detection methods. Similarly, Timisit et al. (2010) monitored rumen temperature with a CUSUM and reported a positive predictive value of 73% for detecting BRD in young bulls. Furthermore, the onset of clinical signs of BRD always

occurred after the CUSUM detected an increase in rumen temperature, the time-lag ranged from 0.5 – 11 d depending on the symptom. A challenge/opportunity with monitoring rumen temperature for detection of BRD, is that rumen temperature can be influenced by other factors than BRD. Previous reports have outlined the value of monitoring rumen temperature for subacute ruminal acidosis, mastitis, parturition and dystocia (AlZahal et al., 2008; Adams et al., 2012; Costa et al., 2016; Kovács et al., 2016). Results from this study and others highlight the value of monitoring rumen temperature for not only BRD detection, but as a general measure of animal health and homeostasis.

Conclusion

Current methods for the detection of BRD are highly specific but not very sensitive. These methods lead to a large proportion of animals contracting sub-clinical disease during the finishing phase of production, which leads to decreased gain, carcass quality and profitability. Results from this study show that deviations in DMI, feeding behaviors and rumen temperature are good indicators of animal health. Furthermore, these measures coupled with SPC procedures can accurately differentiate between healthy and challenged animals. Sensitivity and specificity of the SPC procedures were an improvement over the current method which employs visual detection of clinical symptoms. Results from this study highlight the value of remote continuously recorded data coupled with robust detection methods.

CHAPTER 8

CONCLUSIONS

Regardless of the advancements made in pharmaceutical products designed to combat BRD, the disease complex continues to negatively impact animal welfare and profitability. Both challenge models effectively stimulated inflammation, upregulated leukocytes and acute phase proteins, synonymous with an acute immune response associated with naturally occurring BRD. Furthermore, these challenge models impacted feeding behaviors similarly to an acute BRD infection. In general, the VB challenge model induced a greater immune response. However, neither of these challenge models created gross signs of morbidity or mortalities. Both models should serve future research endeavors as models for BRD without risking loss of life or creating distress for test subjects.

Supplementation with LY minimally impacted performance, physiologic or behavior responses in the challenge studies. When animals were challenged with MH only, steers supplemented with LY had improved ADG and feed efficiency post experimental challenge and these improvements continued after cessation of supplementation throughout the duration of the study. However, there were no differences in animal performance or feed efficiency due to LY when animals were challenge with the VB challenge model. Supplementation with LY did impact cortisol concentration when steers were challenged with MH only. In the VB challenge LY supplementation increased neutrophils and monocytes and, reduced haptoglobin

concentration. These results suggest that LY supplementation impacts the functionality of the immune system in a beneficial manner; although the impacts on overall animal performance is unclear.

Current methods for the detection of BRD are highly specific but not very sensitive. These methods lead to a large proportion of animals contracting sub-clinical disease during the finishing phase of production, which leads to decreased gain, carcass quality and profitability. In the chapters that evaluate the use of SPC algorithms to detect either naturally occurring BRD or the challenge models the SPC algorithms were more accurate than current estimates of employing visual appraisal. In general, DMI, feeding behaviors and RUT were more accurate than the accelerometer based traits. In the naturally occurring disease outbreak the PCA derived factors were more accurate than the phenotypic traits. Furthermore, the Shewhart analysis was more accurate at detecting the disease challenges than the naturally occurring BRD outbreak. In application the PCA derived traits coupled with the CUSUM analysis may be more accurate at identifying sub-clinical BRD. These results illustrate the value of electronic data collection systems coupled with robust prediction procedures for the beef industry, through identification of morbid animals prior to overt clinical signs of disease.

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APPENDIX

FIGURES AND TABLES

Chapter 2 Figures and Tables

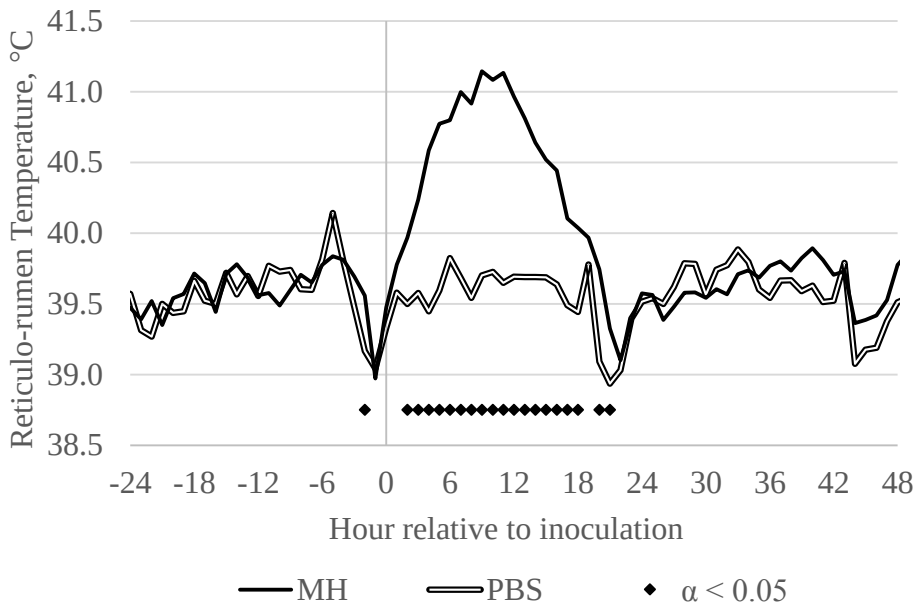

Figure 1. Model-adjusted least squares mean rumen temperature (RUT) by hour relative to experimental inoculation with *Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS; negative control).
The model included effects for study hour and repeated measures on individual steers with initial exit velocity as a covariate. ♦Significant differences ($P < 0.05$) between treatments groups within study hour. The interaction between trial hour and treatment group was significant ($P < 0.001$)**.**

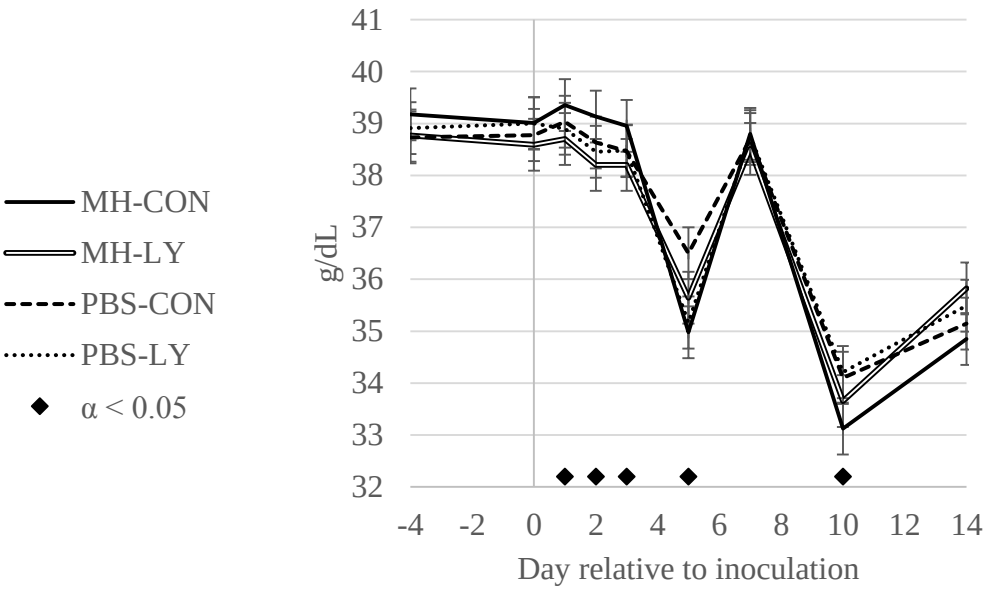

Figure 2. Model-adjusted least squares mean corpuscular hemoglobin concentration (MCHC) for the interaction ($P < 0.01$) of day (relative to inoculation) x inoculation type (*Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS; negative control)) x dietary treatment (roughage-based diet without (CON) or with added live yeast (LY; *Saccharomyces cerevisiae boulardii* strain I-1079 at 25 g/hd/d; Proternative Advantage; Lallemand Animal Nutrition)).
The model included effects for study day and repeated measures on individual steers with initial exit velocity as a covariate. ♦Significant differences ($P < 0.05$) between treatments groups within study day. Multiple comparisons for days that differ are in Table 3.

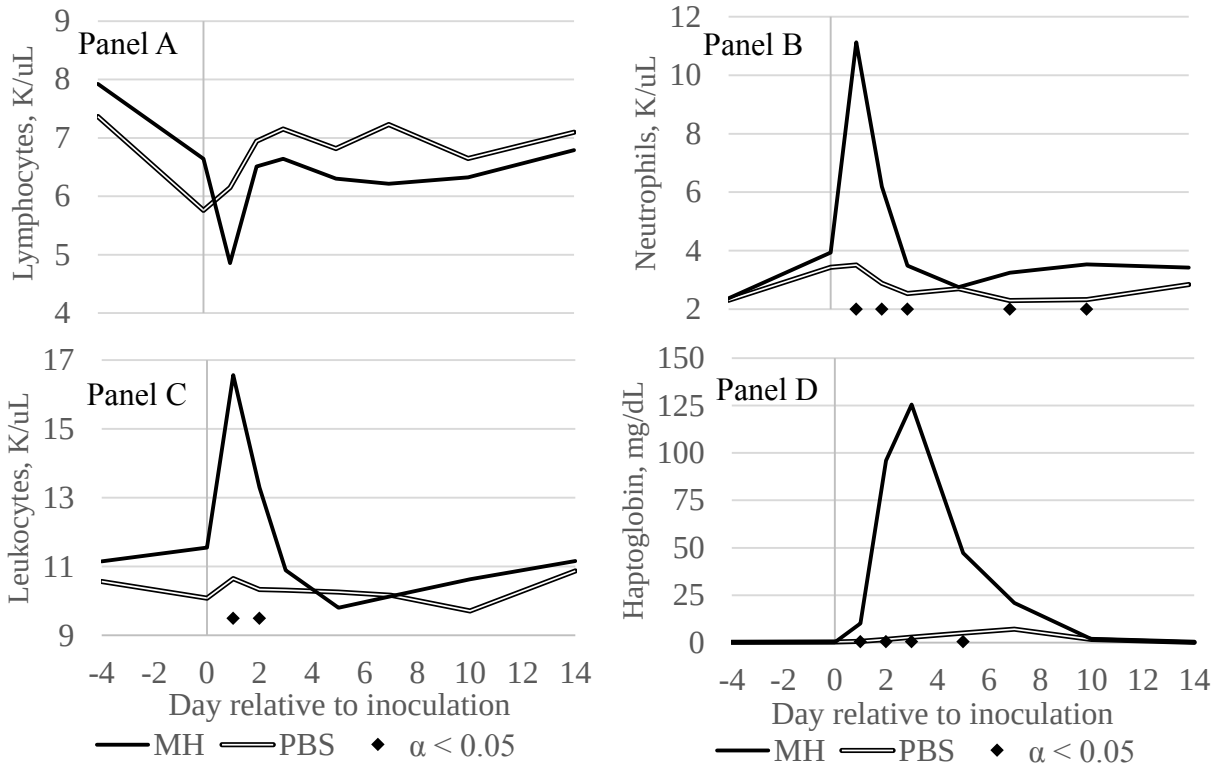

Figure 3. Model-adjusted least squares mean lymphocytes (A), neutrophils (B), leukocytes (C) and haptoglobin (D) by day relative to experimental inoculation with *Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS; negative control).

The model included effects for study day and repeated measures on individual steers with initial exit velocity as a covariate. ♦Significant differences ($P < 0.05$) between treatments groups within trial day. The inoculation x day interaction was significant ($P < 0.01$).

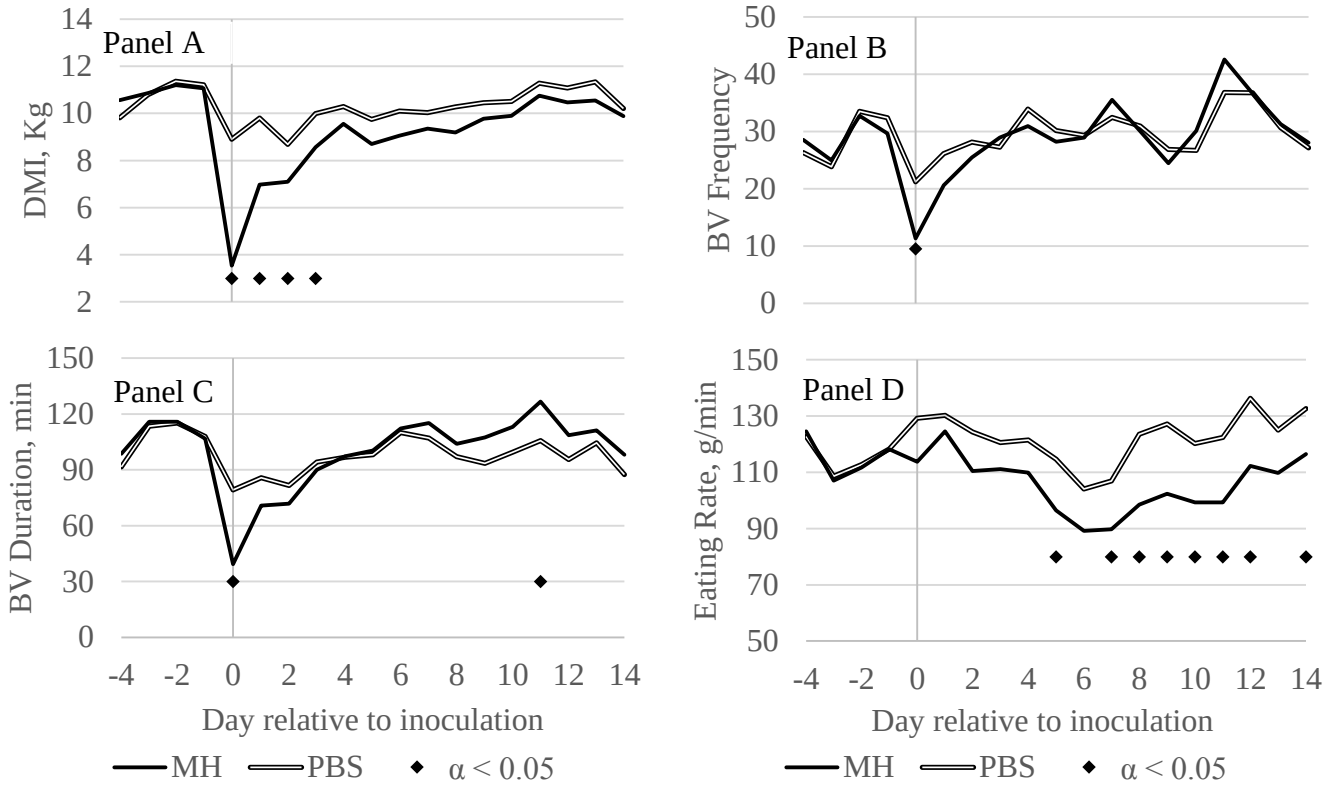

Figure 4. Model-adjusted least squares mean DMI (A), bunk visit (BV) frequency (events/d; B), BV duration (C) and eating rate (D) by day relative to experimental inoculation with *Mannheimia haemolytica* (MH) or phosphate buffer solution (PBS; negative control).

The model included effects for study day and repeated measures on individual steers with initial exit velocity as a covariate. ♦Significant differences ($P < 0.05$) between treatments groups within trial day. The inoculation x day interaction was significant ($P < 0.01$).

Table 1. Main effects of live-yeast (LY) treatment and inoculation type on rectal and rumen temperature pre- and post-challenge with *Mannheimia haemolytica* (MH)

	Dietary treatment				Inoculation				Interactions			
Item	Con	LY	SE	P-Value	MH	PBS	SE	P-Value	In*Diet	In*Day	Diet*Day	In*Diet*Day
No. of Steers	18	18	--	--	18	18	--	--				
Rectal Temperature ℃												
All days	39.46	39.64	0.04	**0.02**	39.54	39.55	0.04	0.82	0.76	**0.01**	**0.02**	0.51
Day -4	39.80	39.86	0.09	0.68	39.70	39.96	0.09	**0.05**	0.55	-	-	-
Day 0	39.55	39.80	0.09	**0.04**	39.63	39.72	0.09	0.49	0.98	-	-	-
Day 1	39.68	39.80	0.14	0.53	39.95	39.54	0.14	**0.04**	0.67	-	-	-
Day 2	39.63	39.67	0.07	0.68	39.69	39.62	0.07	0.52	0.10	-	-	-
Day 3	39.35	39.48	0.10	0.22	39.36	39.46	0.10	0.35	0.38	-	-	-
Day 5	39.44	39.60	0.11	0.17	39.53	39.51	0.11	0.85	0.37	-	-	-
Day 7	39.50	39.86	0.10	**0.01**	39.64	39.73	0.10	0.33	0.87	-	-	-
Day 10	39.37	39.63	0.12	**0.03**	39.50	39.50	0.12	0.96	0.30	-	-	-
Day 14	39.57	39.75	0.11	0.12	39.66	39.67	0.11	0.96	0.96	-	-	-
Reticulo-rumen Temperature ℃												
All days	39.51	39.54	0.04	0.64	39.59	39.46	0.04	**0.03**	0.97	0.07	0.45	0.91
Day -4	39.40	39.43	0.09	0.76	39.41	39.41	0.09	0.99	0.99	-	-	-
Day 0	39.83	39.86	0.13	0.84	40.09	39.61	0.13	**0.01**	0.77	-	-	-
Day 1	39.72	39.68	0.14	0.79	39.84	39.57	0.14	0.07	0.52	-	-	-
Day 2	39.73	39.64	0.09	0.47	39.80	39.57	0.09	0.09	0.52	-	-	-
Day 3	39.52	39.47	0.09	0.59	39.53	39.46	0.09	0.42	0.74	-	-	-
Day 5	39.55	39.55	0.09	0.99	39.61	39.48	0.09	0.15	0.92	-	-	-
Day 7	39.37	39.54	0.10	0.09	39.51	39.40	0.10	0.27	0.82	-	-	-
Day 10	39.45	39.51	0.07	0.57	39.48	39.47	0.07	0.92	0.85	-	-	-
Day 14	39.63	39.63	0.09	0.98	39.64	39.61	0.09	0.77	0.93	-	-	-

*MH = *Mannheimia haemolytica*, PBS = phosphate buffer solution, LY = live yeast (*Saccharomyces cerevisiae boulardii*), CON = control diet and In = inoculation.

Table 2. Main effects of dietary treatment and inoculation on complete blood cell count and haptoglobin concentrations

	Dietary treatment				Inoculation				Interactions			
Item	CON	LY	SE	P-Value	MH	PBS	SE	P-Value	In*Diet	In*Day	Diet*Day	In*Diet*Day
N	162	162	--	--	162	162	--	--	--	--	--	--
Eosinophil, K/uL	0.31	0.23	0.05	0.14	0.25	0.29	0.05	0.43	0.69	0.19	0.85	0.28
Basophils, K/uL	0.05	0.05	0.01	0.75	0.05	0.06	0.01	0.20	0.11	0.11	**0.04**	0.17
Lymphocytes, K/uL	6.61	6.65	0.28	0.92	6.47	6.80	0.28	0.41	0.76	**0.01**	0.76	0.16
Neutrophils, K/uL	3.62	3.59	0.19	0.90	4.45	2.76	0.19	**0.01**	0.40	**0.01**	0.15	0.60
Monocytes, K/uL	0.50	0.41	0.04	*0.06*	0.50	0.41	0.04	*0.06*	0.71	0.54	0.67	0.80
Hematocrit, %	33.9	34.2	0.52	0.63	33.8	34.2	0.52	0.55	0.11	0.41	0.65	0.44
Hemoglobin, g/dL	12.7	12.7	0.25	0.96	12.5	12.8	0.25	0.27	**0.04**	0.23	0.24	0.70
MCH, pg	15.7	15.9	0.21	0.63	15.7	15.8	0.21	0.76	0.25	0.98	*0.09*	0.48
MCV, fL	42.0	42.5	0.54	0.56	42.2	42.3	0.54	0.89	0.22	0.08	0.37	*0.09*
MCHC, g/dl	37.5	37.4	0.12	0.36	37.4	37.5	0.12	0.35	0.78	*0.07*	0.14	**0.01**
Erythrocytes, M/uL	8.06	8.05	0.17	0.94	8.03	8.09	0.17	0.70	0.81	*0.09*	*0.07*	0.67
Leukocytes, K/uL	11.1	10.9	0.38	0.79	11.7	10.3	0.38	**0.02**	0.42	**0.01**	0.16	0.66
Platelets, K/uL	523	537	29.0	0.63	535	526	29.0	0.76	0.21	**0.01**	0.69	*0.07*
Haptoglobin, mg/dL	17.9	17.8	6.29	0.99	33.6	2.10	6.29	**0.01**	0.57	**0.01**	0.50	0.52
Cortisol, ng/mL	26.1	34.4	3.20	**0.02**	28.5	32.0	3.20	0.27	0.46	*0.09*	0.30	0.87

*MH = *Mannheimia haemolytica*, PBS = phosphate buffer solution, LY = live yeast (*Saccharomyces cerevisiae boulardii*), CON = control diet, In = inoculation, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume and MCHC = mean corpuscular hemoglobin concentration.

Table 3. Mean corpuscular hemoglobin concentration (MCHC) by day

Treatment	-4	0	1	2	3	5	7	10	14
MH-CON	39.2	39.0	39.4^{a}	39.1^{a}	39.0^{a}	35.0^{a}	38.8	33.1^{a}	34.9
MH-LY	38.8	38.6	38.7^{b}	38.2^{b}	38.2^{b}	35.6^{ab}	38.5	33.7^{ab}	35.8
PBS-CON	38.7	38.8	39.0^{ab}	38.6^{ab}	38.5^{ab}	36.5^{b}	38.7	34.1^{b}	35.1
PBS-LY	38.9	39.0	38.9^{ab}	38.5^{b}	38.5^{ab}	35.2^{ab}	38.8	34.2^{b}	35.5

*MH = *Mannheimia haemolytica*, PBS = phosphate buffer solution, CON = control diet and LY = Live Yeast. *Treatments with different superscripts by day differ $P < 0.05$

Chapter 3 Figures and Tables

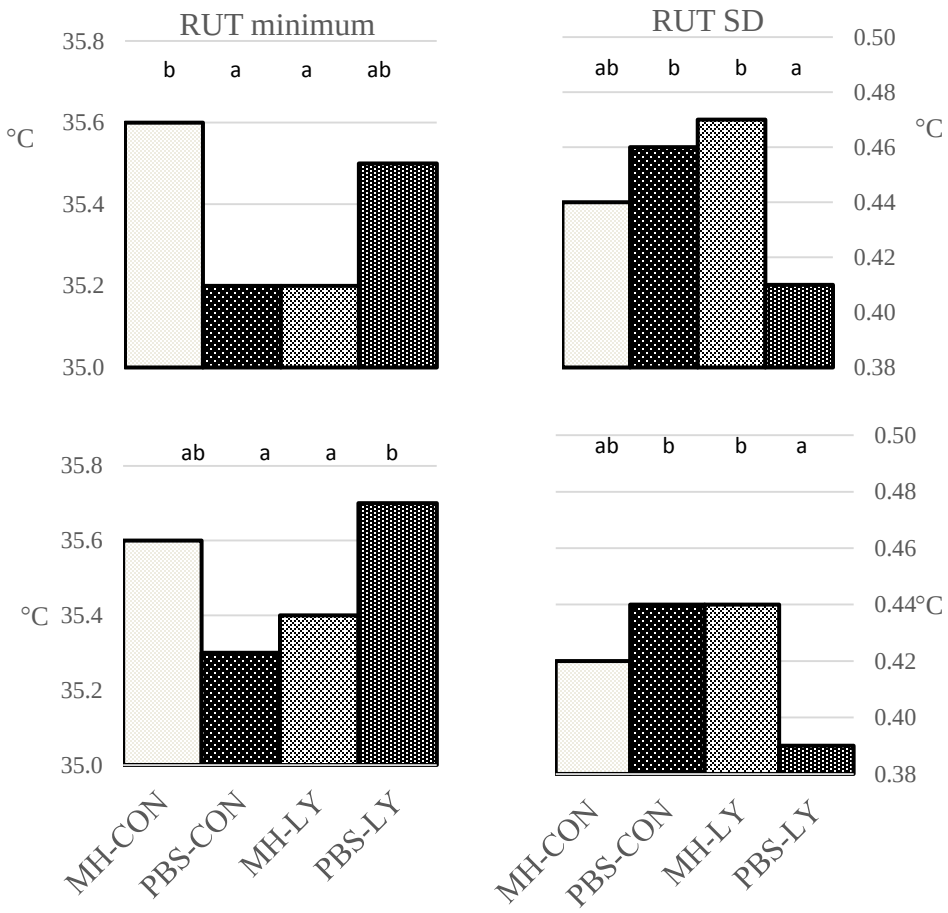

Figure 5. Comparison of sub-class means for minimum rumen temperature (RUT; left) and SD of RUT (right) during days 28 through 56 (above) and 28 through 84 (below). * CON = dietary control, LY = dietary treatment of *Saccharomyces cerevisiae boulardii*, MH = endoscopic inoculation with *Mannheimia haemolytica* and PBS = inoculation with phosphate buffer solution. Columns within a panel with different superscripts differ ($P < 0.05$).

Table 4. Effects of live-yeast (LY) and inoculation type on performance, feed efficiency, and feeding behavior traits in growing steers during the first 28 d of the trial with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	18	18	--	--	18	18	--	--	--
Performance and Feed Efficiency									
Initial BW, kg	349	356	8.0	0.41	350	355	7.9	0.58	0.89
BW (day 28), kg	398	398	8.7	0.95	396	400	8.5	0.64	0.99
ADG, kg/d	1.74	1.52	0.08	**0.01**	1.63	1.62	0.08	0.85	0.57
DMI, kg/d	11.2	10.8	0.32	0.16	10.7	11.2	0.03	0.13	0.86
Daily DMI Var, kg	1.93	1.93	0.13	0.99	2.06	1.80	0.13	**0.05**	0.63
DMI, % BW	3.01	2.85	0.07	**0.05**	2.88	2.98	0.07	0.16	0.88
G:F ratio	0.16	0.14	0.01	**0.01**	0.15	0.14	0.01	0.15	0.37
Bunk Visit (BV) Traits									
BV frequency, events/d	54.5	47.1	3.67	**0.05**	51.1	50.4	3.59	0.85	0.74
BV duration, min/d	118	122	7.60	0.67	122	119	7.57	0.74	0.49
Head down duration, min/d	66.4	57.8	8.10	0.29	60.2	64.0	7.92	0.64	0.73
NFI duration, min/d	1260	1250	10.6	0.29	1253	1257	10.7	0.71	0.79
NFI max, min/d	494	454	27.5	0.16	478	469	26.9	0.74	0.37
NFI SD, min/d	84.4	87.4	5.31	0.57	86.9	85.0	5.19	0.71	0.43
Meal Event traits									
Meal criterion, min	5.25	6.18	1.19	0.44	5.62	5.81	1.10	0.86	0.47
Meal frequency, events/d	15.2	13.7	1.47	0.33	14.0	14.8	1.44	0.58	0.92
Meal length, min/event	12.7	14.9	2.04	0.29	14.4	13.3	2.00	0.60	0.62
Meal intake, kg	0.94	1.02	0.11	0.43	0.98	0.98	0.11	0.97	0.78
Meal duration, min/d	169	170	10.7	0.92	173	167	10.4	0.55	0.97
Eating rate, g/min	97.0	91.0	5.04	0.24	91.7	96.3	4.93	0.35	0.52
Time to bunk, min	19.3	38.7	7.26	**0.01**	27.6	30.4	7.10	0.69	0.26

Table 5. Effects of live-yeast (LY) and inoculation treatments on accelerometer-based behavior traits and rumen temperature in growing steers during the first 28 d of the trial with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	15	18	--	--	16	15	--	--	--
Accelerometer-based Behaviors									
Ingestion, min/d	205	214	13.5	0.53	216	203	13.2	0.35	0.10
Rumination, min/d	414	402	20.4	0.58	417	399	19.9	0.36	0.19
Rest, min/d	528	539	21.1	0.60	527	541	20.5	0.51	0.38
Other activity, min/d	201	191	11.6	0.41	192	199	11.3	0.57	0.58
Over activity, min/d	92.5	93.9	5.32	0.80	87.8	98.6	5.18	**0.05**	0.18
Standing, min/d	901	916	13.3	0.26	900	918	12.9	0.18	0.73
No. of Steers	18	18	--	--	18	18	--	--	--
Rumen Temperature									
Average, C°	39.3	39.4	0.05	0.08	39.3	39.3	0.05	0.67	0.69
Minimum, C°	34.3	34.3	0.14	0.96	34.3	34.3	0.14	0.96	0.60
Maximum, C°	40.2	40.2	0.06	0.33	40.2	40.2	0.06	0.35	0.75
SD, C°	0.52	0.51	0.02	0.43	0.52	0.51	0.02	0.69	0.34

* The reduced number of measurements for the accelerometer-based behaviors is due to sensor failure.

Table 6. Effects of live-yeast (LY) and inoculation treatments on performance, feed efficiency, and feeding behavior traits in growing steers during days 28-56 with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	18	18	--	--	18	18	--	--	--
Performance and Feed Efficiency									
Initial BW (day 28), kg	389	389	8.90	0.65	384	394	8.70	0.30	0.89
BW (day 56), kg	421	431	10.6	0.36	419	433	10.3	0.17	0.86
ADG, kg/d	1.13	1.50	0.15	**0.02**	1.22	1.41	0.15	0.19	0.87
DMI, kg/d	10.1	10.3	0.45	0.72	9.8	10.6	0.44	*0.06*	0.54
Daily DMI Var kg	2.05	1.81	0.17	0.16	2.30	1.6	0.16	**0.01**	0.57
DMI, % BW	2.49	2.51	0.08	0.83	2.42	2.58	0.08	*0.06*	0.42
G:F ratio	0.11	0.15	0.02	**0.01**	0.12	0.13	0.02	0.40	0.64
Bunk Visit (BV) Traits									
BV frequency, events/d	34.4	30.8	2.7	0.20	32.9	32.4	2.60	0.85	0.32
BV duration, min/d	101	102	6.36	0.84	104	99	6.22	0.47	0.75
Head down duration, min/d	60.2	52.7	6.29	0.24	56.1	56.8	6.15	0.90	0.87
NFI duration, min/d	1255	1317	9.54	**0.01**	1283	1289	9.32	0.52	0.75
NFI max, min/d	485	447	27.3	0.18	480	447	26.7	0.31	0.53
NFI SD, min/d	100	100	6.77	0.94	104	96	6.62	0.27	0.76
Meal Event traits									
Meal criterion, min	6.93	6.18	1.15	0.52	6.70	6.41	1.11	0.79	0.77
Meal frequency, events/d	11.4	12.0	0.96	0.57	11.3	12.1	0.94	0.40	0.72
Meal length, min/event	12.9	11.9	1.32	0.44	12.9	11.9	1.30	0.43	0.64
Meal intake, kg	1.04	1.04	0.09	0.99	1.02	1.05	0.09	0.76	0.31
Meal duration, min/d	138	130	8.14	0.31	138	130	7.97	0.36	0.83
Eating rate, g/min	103	103	5.50	0.93	96	110	5.40	**0.02**	0.83
Time to bunk, min	27.6	36.0	7.25	0.25	35.5	28.1	7.09	0.31	0.72

Table 7. Effects of live-yeast (LY) and inoculation treatments on accelerometer-based behavior traits and rumen temperature in growing steers during days 28 through 56 of the trial with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	15	18	--	--	16	15	--	--	--
Accelerometer-based Behaviors									
Ingestion, min/d	183	179	13.7	0.74	179	184	13.4	0.69	0.11
Rumination, min/d	317	335	22.0	0.40	324	328	21.4	0.83	0.66
Rest, min/d	621	620	30.0	0.98	616	625	29.2	0.75	0.60
Other activity, min/d	227	212	14.8	0.30	226	212	14.5	0.35	0.82
Over activity, min/d	92.5	94.4	9.07	0.83	96.3	90.5	8.83	0.52	0.96
Standing, min/d	919	949	18.4	0.12	928	940	17.9	0.49	0.25
No. of Steers	18	18	--	--	18	18	--	--	--
Rumen Temperature									
Average, C°	39.5	39.6	0.07	0.19	39.6	39.5	0.06	0.18	0.92
Minimum, C°	35.4	35.4	0.13	0.80	35.4	35.4	0.13	0.73	**0.01**
Maximum, C°	40.3	40.4	0.08	0.34	40.5	40.3	0.07	**0.02**	0.96
SD, C°	0.45	0.44	0.01	0.40	0.45	0.43	0.01	0.11	**0.01**

* The reduced number of measurements for the accelerometer-based behaviors is due to sensor failure.

Table 8. Effects of live-yeast (LY) and inoculation treatments on performance, feed efficiency, and feeding behavior traits in growing steers during days 28-84 (56 d following inoculation) with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	18	18	--	--	18	18	--	--	--
Performance and Feed Efficiency									
Initial BW (day 28), kg	388	389	8.90	0.94	384	393	8.90	0.29	0.24
BW (day 56), kg	463	476	11.5	0.27	461	478	11.50	0.15	0.29
ADG, kg/d	1.33	1.56	0.11	**0.05**	1.39	1.50	0.11	0.25	0.79
DMI, kg/d	10.3	10.5	0.39	0.61	10.1	10.8	0.39	*0.07*	0.11
Daily DMI Var kg	1.96	1.76	0.17	0.25	2.15	1.57	0.17	**0.01**	0.67
DMI, % BW	2.42	2.43	0.08	0.81	2.38	2.48	0.08	*0.09*	0.13
G:F ratio	0.13	0.15	0.01	**0.02**	0.14	0.14	0.01	0.78	0.55
Bunk Visit (BV) Traits									
BV frequency, events/d	32.8	30.9	2.22	0.39	32.3	31.4	2.22	0.66	0.18
BV duration, min/d	94.4	95.5	5.82	0.85	97.1	92.8	5.82	0.46	0.99
Head down duration, min/d	56.2	47.6	6.06	0.17	51.2	52.6	6.06	0.81	0.75
NFI duration, min/d	1304	1329	6.45	**0.05**	1314	1320	6.45	0.34	0.91
NFI max, min/d	482	445	25.5	0.16	474	453	25.5	0.41	0.43
NFI SD, min/d	103	100	6.31	0.65	104	99	6.31	0.44	0.45
Meal Event traits									
Meal criterion, min	6.72	6.42	0.85	0.72	7.00	6.13	0.82	0.30	0.82
Meal frequency, events/d	11.4	12.0	0.94	0.57	11.1	12.3	0.94	0.55	0.62
Meal length, min/event	11.9	11.5	1.13	0.68	12.5	10.9	1.13	0.14	0.89
Meal intake, kg	1.01	1.07	0.09	0.99	1.08	1.05	0.09	0.75	0.26
Meal duration, min/d	128	125	7.82	0.63	131	122	7.82	0.26	0.51
Eating rate, g/min	113	112	5.89	0.91	106	119	5.89	**0.03**	0.63
Time to bunk, min	23.0	27.6	5.60	0.42	29.7	21.0	5.6	0.12	0.71

Table 9. Effects of live-yeast (LY) and inoculation treatments on accelerometer-behavior traits and rumen temperature in growing steers during days 28 through 84 (56 d following inoculation) with the effect of initial exit velocity as a covariate

	Dietary treatment				Inoculation				
Item	CON	LY	SE	P-Val	MH	PBS	SE	P-Val	Interaction
No. of Steers	15	18	--	--	16	15	--	--	--
Accelerometer-based Behaviors									
Ingestion, min/d	198	191	11.5	0.54	192	196	11.2	0.74	0.08
Rumination, min/d	306	316	22.8	0.65	315	307	22.2	0.73	0.55
Rest, min/d	631	638	33.8	0.85	623	646	32.9	0.48	0.83
Other activity, min/d	218	208	14.9	0.48	219	207	14.5	0.39	0.98
Over activity, min/d	86.8	87.8	8.20	0.90	90.9	83.7	7.95	0.37	0.92
Standing, min/d	925	939	8.26	0.10	932	933	8.05	0.88	0.50
No. of Steers	18	18	--	--	18	18	--	--	--
Rumen Temperature									
Average, C°	39.4	39.5	0.06	**0.05**	39.5	39.5	0.06	0.48	0.93
Minimum, C°	35.5	35.6	0.14	0.47	35.5	35.5	0.13	0.85	**0.02**
Maximum, C°	40.3	40.3	0.06	0.23	40.4	40.2	0.06	*0.08*	0.90
SD, C°	0.43	0.42	0.01	0.24	0.43	0.42	0.01	0.25	**0.01**

* The reduced number of measurements for the accelerometer-based behaviors is due to sensor failure.

Chapter 4 Figures and Tables

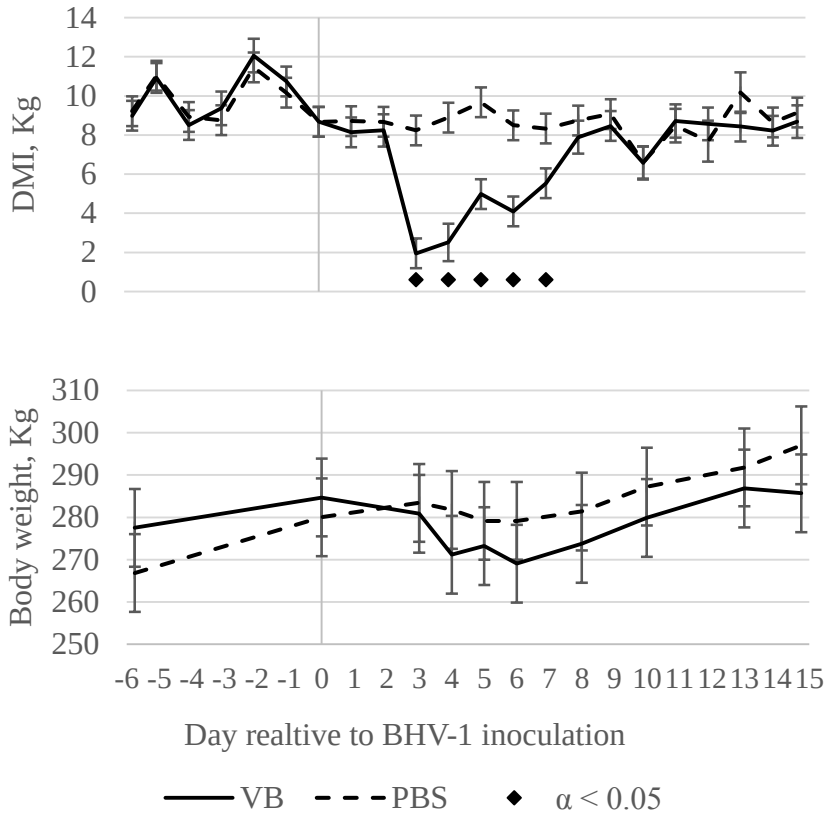

Figure 6. Model-adjusted least squares mean estimates for DMI (above) and body weight (below) by day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control).
The model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate.
♦Significant differences ($P < 0.05$) between treatments groups within study day. The inoculation x day interaction was significant ($P < 0.001$) for both DMI and body weight.

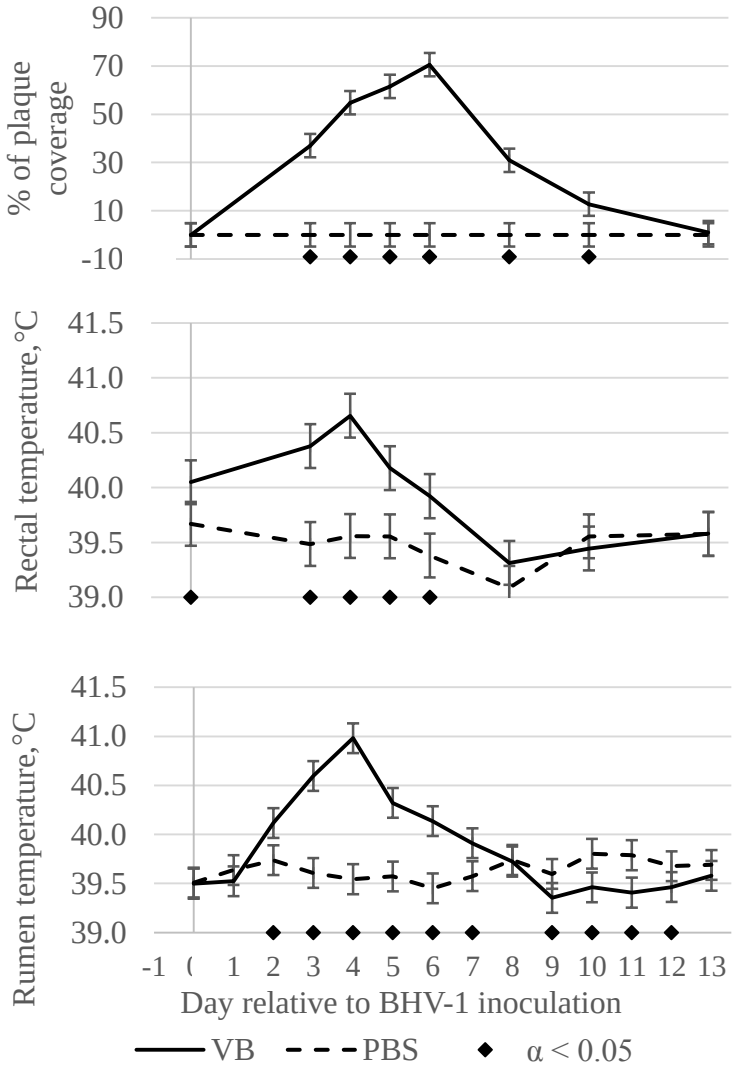

Figure 7. Model-adjusted least squares mean estimates for nasal lesions (top), rectal temperature (middle) and rumen temperature (RUT; bottom) by day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control).
Model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate.
♦Significant differences ($P < 0.05$) between treatments groups within study day. The inoculation x day interaction was significant for all dependent variables ($P < 0.001$).

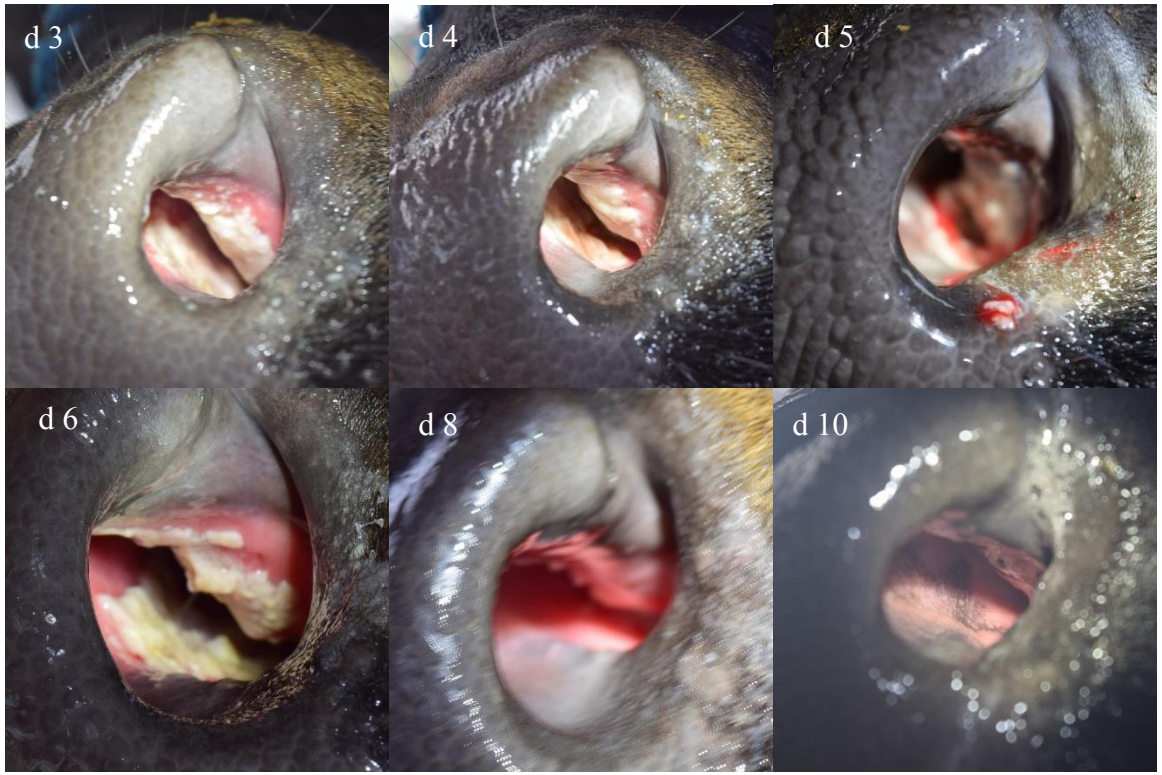

Figure 8. Progression of bovine herpes virus-1 plaques, beginning on day 3 following intranasal inoculation.

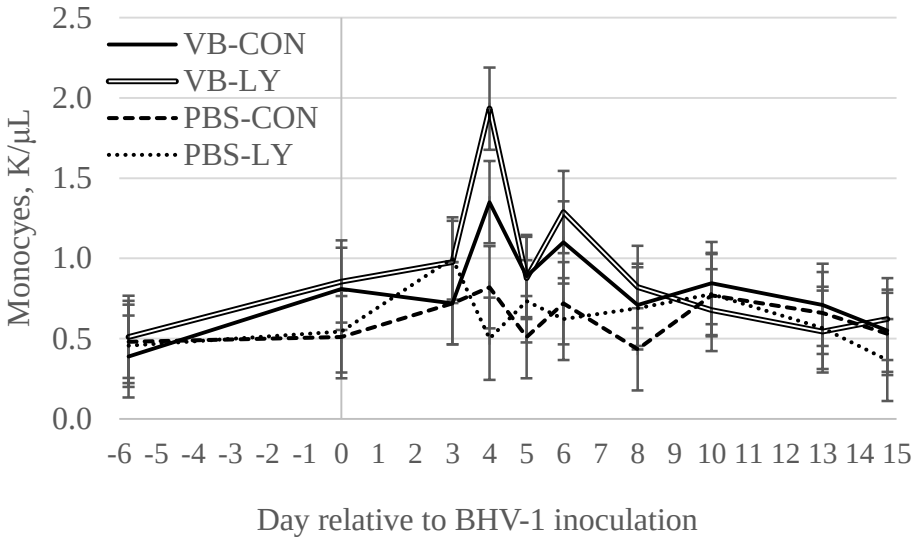

Figure 9. Model-adjusted least squares mean estimates of monocytes for the interaction of inoculation x diet x day. With day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control). The model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate. The inoculation x diet x day interaction was significant ($P < 0.001$). CON = control diet and LY = supplementation with *Saccharomyces cerevisiae boulardii*.

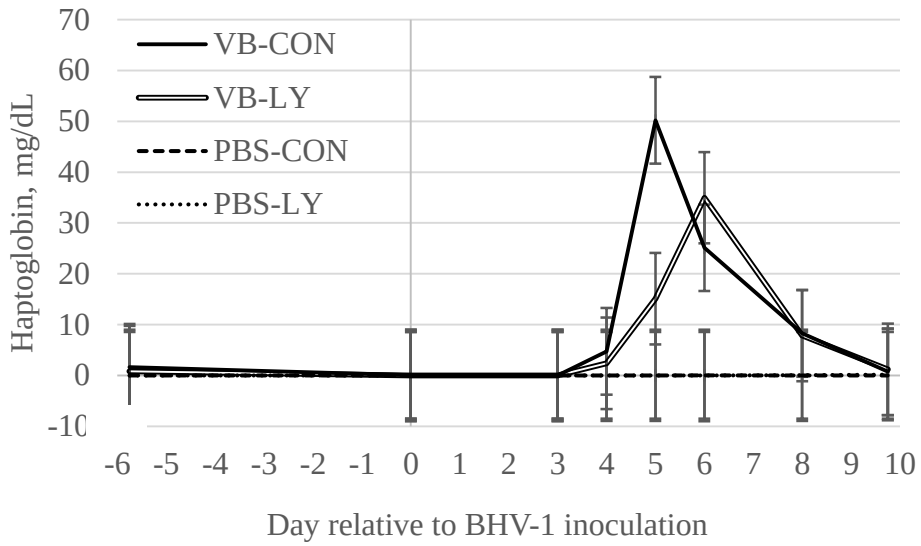

Figure 10. Model-adjusted least squares mean estimates of haptoglobin for the interaction of inoculation x diet x day. With day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control).
The model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate. The inoculation x diet x day interaction was significant ($P < 0.02$). CON = control diet and LY = supplementation with *Saccharomyces cerevisiae boulardii*.

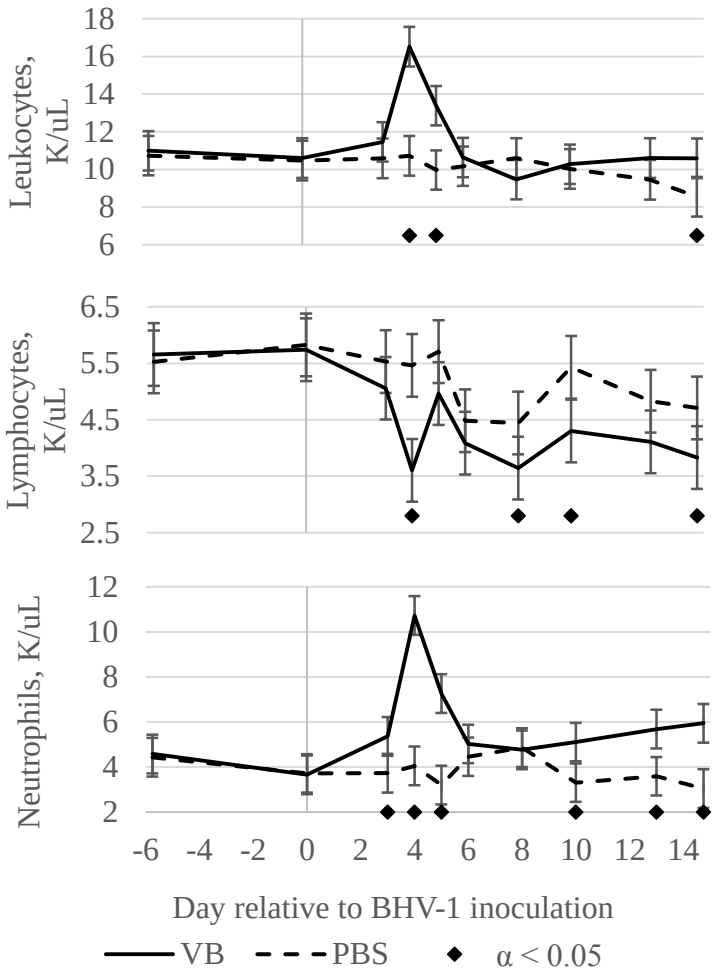

Figure 11. Model-adjusted least squares mean estimates for leukocytes (top), lymphocytes (middle) and neutrophils (bottom) by day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control).
Model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate.
♦Significant differences ($P < 0.05$) between treatments groups within study day. The inoculation x day interaction was significant for all dependent variables ($P < 0.001$).

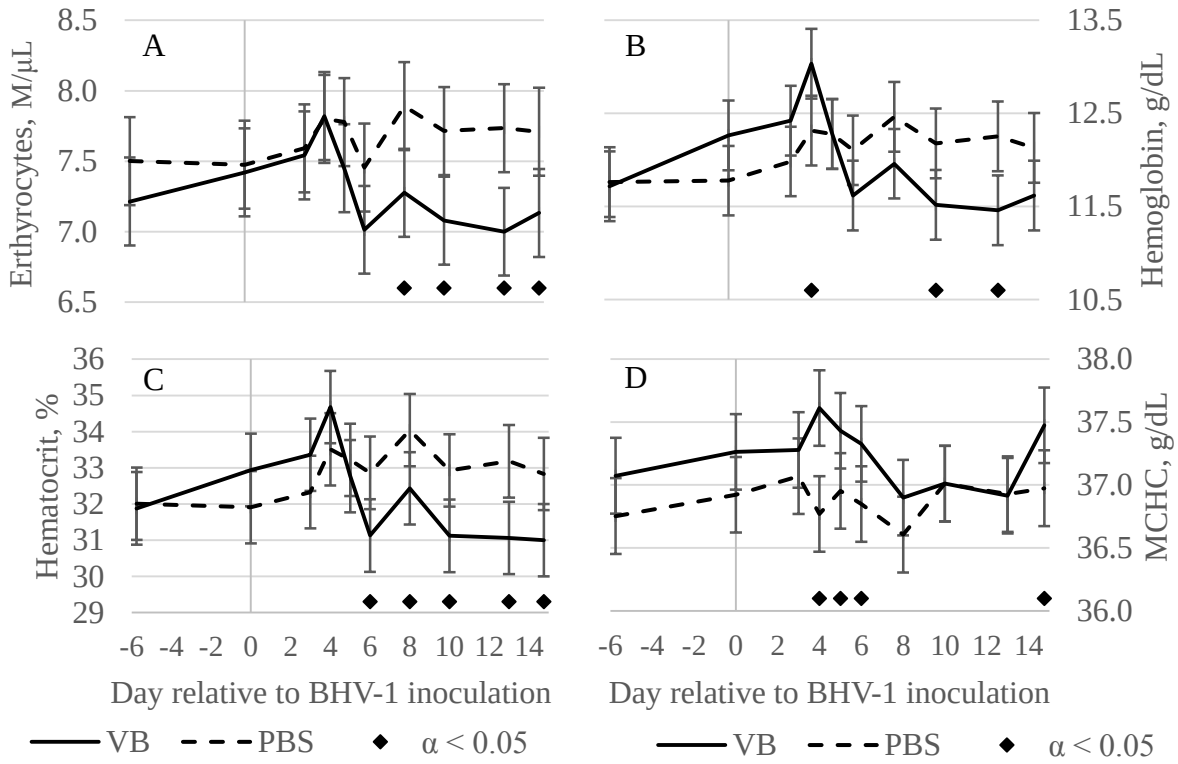

Figure 12. Model-adjusted least squares mean estimates for erythrocytes (Panel A), hemoglobin (Panel B), hematocrit (Panel C) and mean corpuscular hemoglobin concentration (MCHC; Panel D) by day relative to experimental inoculation with viral-bacterial (VB; bovine herpes virus-1 (BHV-1) on day 0 followed by *Mannheimia haemolytica* on day 3) challenge or phosphate buffer solution (PBS; negative control).
The model included effects for study day and repeated measures on individual heifers with pre-ADG as a covariate.
♦Significant differences ($P < 0.05$) between treatments groups within study day. The inoculation x day interaction was significant for all dependent variables ($P < 0.001$).

Table 10. Main effects of dietary and inoculation treatments on hemogram constituents, haptoglobin and cortisol concentrations

	Dietary treatment				Inoculation				P-Values			
Item	Con	LY	SE	P-Value	VB	PBS	SE	P-Value	In*Diet	In*D	Diet*D	In*Diet*D
N	20	18	--	--	19	19	--	--	--	--	--	--
Eosinophil, K/uL	0.33	0.32	0.04	0.83	0.28	0.37	0.05	0.09	0.21	0.09	0.68	0.24
Lymphocytes, K/uL	5.03	4.88	0.19	0.44	4.85	5.06	0.19	0.27	0.25	**0.01**	1.00	0.81
Neutrophils, K/uL	4.40	5.12	0.29	**0.02**	5.56	3.96	0.29	**0.01**	0.08	**0.01**	0.46	0.59
Monocytes, K/uL	0.70	0.75	0.05	0.29	0.83	0.62	0.05	**0.01**	0.58	**0.01**	0.72	**0.01**
Leukocytes, K/uL	10.5	11.1	0.47	0.16	11.5	10.1	0.47	**0.05**	0.60	**0.01**	0.43	0.28
Hemoglobin, g/dL	12.0	12.1	0.19	0.72	12.1	11.9	0.19	0.33	0.99	**0.01**	0.33	0.16
Platelets, K/uL	637	627	33.5	0.77	634	630	33.5	0.91	0.89	**0.01**	0.96	0.94
Erythrocytes, M/uL	7.54	7.46	0.17	0.59	7.50	7.50	0.17	0.95	0.42	**0.01**	0.84	0.09
Hematocrit, %	0.33	0.33	0.01	0.90	0.33	0.32	0.01	0.27	1.00	**0.01**	0.57	0.06
MCH, pg	15.9	16.2	0.24	0.15	16.2	15.9	0.24	0.17	0.09	0.68	0.76	0.78
MCV, fL	43.3	43.9	0.86	0.44	44.1	43.2	0.86	0.31	0.24	0.10	0.91	0.61
MCHC, g/dl	36.8	37.0	0.12	0.16	36.9	36.9	0.12	0.53	0.88	**0.03**	0.61	0.48
Haptoglobin, mg/dl	5.67	3.91	1.74	0.32	9.56	0.02	1.74	**0.01**	0.31	**0.01**	**0.02**	**0.02**
Cortisol, ng/ml	24.0	27.2	3.23	0.33	25.8	25.3	3.94	0.90	0.24	0.29	0.79	0.70

VB= viral-bacterial challenge which consisted of inoculation with bovine herpes virus-1 followed by *Mannheimia haemolytica*, PBS = phosphate buffer solution (negative control), LY = supplementation with *Saccharomyces cerevisiae boulardii*, CON = control (negative control), In = inoculation treatment, MCH = mean corpuscular hemoglobin, MCV = mean cell volume and MCHC = mean corpuscular hemoglobin concentration.

Table 11. Least square mean estimates for monocytes following experimental viral-bacterial challenge

Day	-6	0	3	4	5	6	8	10	13	15
VB-CON	0.39	0.81	0.72	1.35_{b}	0.89_{b}	1.10_{b}	0.71_{ab}	0.85	0.71	0.55
VB-LY	0.51	0.86	0.98	1.93_{c}	0.88_{b}	1.29_{b}	0.82_{b}	0.68	0.54	0.62
PBS-CON	0.48	0.51	0.72	0.82_{a}	0.51_{a}	0.72_{a}	0.43_{a}	0.77	0.66	0.53
PBS-LY	0.46	0.54	1.00	0.50_{a}	0.73_{ab}	0.62_{a}	0.69_{ab}	0.78	0.57	0.37

VB= viral-bacterial challenge which consisted of inoculation with bovine herpes virus-1 followed by *Mannheimia haemolytica*, PBS = phosphate buffer solution (negative control), LY = supplementation with *Saccharomyces cerevisiae boulardii* and CON = control (negative control).
Interaction of diet x inoculation x day is significant at $P < 0.01$.
Within column, rows with different subscripts differ ($P < 0.05$).
Presented values are in K cells/μL.

Table 12. Least square mean estimates for haptoglobin following experimental viral-bacterial challenge

Day	-6	0	3	4	5	6	8	10
VB-CON	1.61	0.03	0.00	4.74	50.19_{c}	25.12_{b}	8.27	0.74
VB-LY	0.79	0.02	0.01	2.39	15.09_{b}	34.95_{b}	7.83	1.19
PBS-CON	0.00	0.00	0.00	0.00	0.00_{a}	0.01_{a}	0.01	0.00
PBS-LY	0.02	0.00	0.00	0.00	0.01_{a}	0.00_{a}	0.03	0.15

VB = viral-bacterial challenge which consisted of inoculation with bovine herpes virus-1 followed by *Mannheimia haemolytica*, PBS = phosphate buffer solution (negative control), LY = supplementation with *Saccharomyces cerevisiae boulardii* and CON = control (negative control).
Interaction of diet x inoculation x day is significant at $P < 0.01$.
Within column, rows with different subscripts differ ($P < 0.05$).
Presented values are in mg/dL.

Chapter 5 Figures and Tables

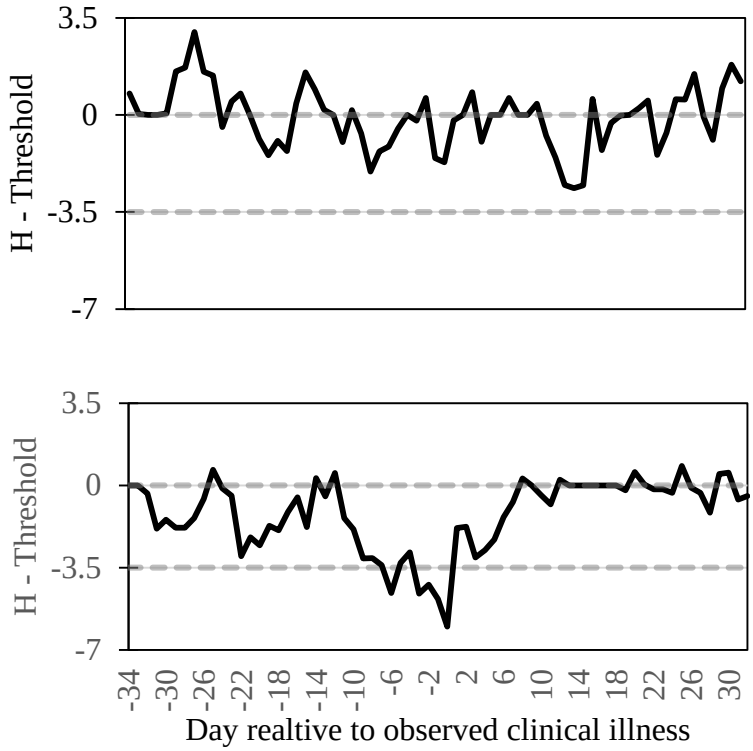

Figure 13. Example of a CUSUM chart for BV frequency that remained within (Above) and exceeded (Below) the control limit of -3.5 H-parameter value.

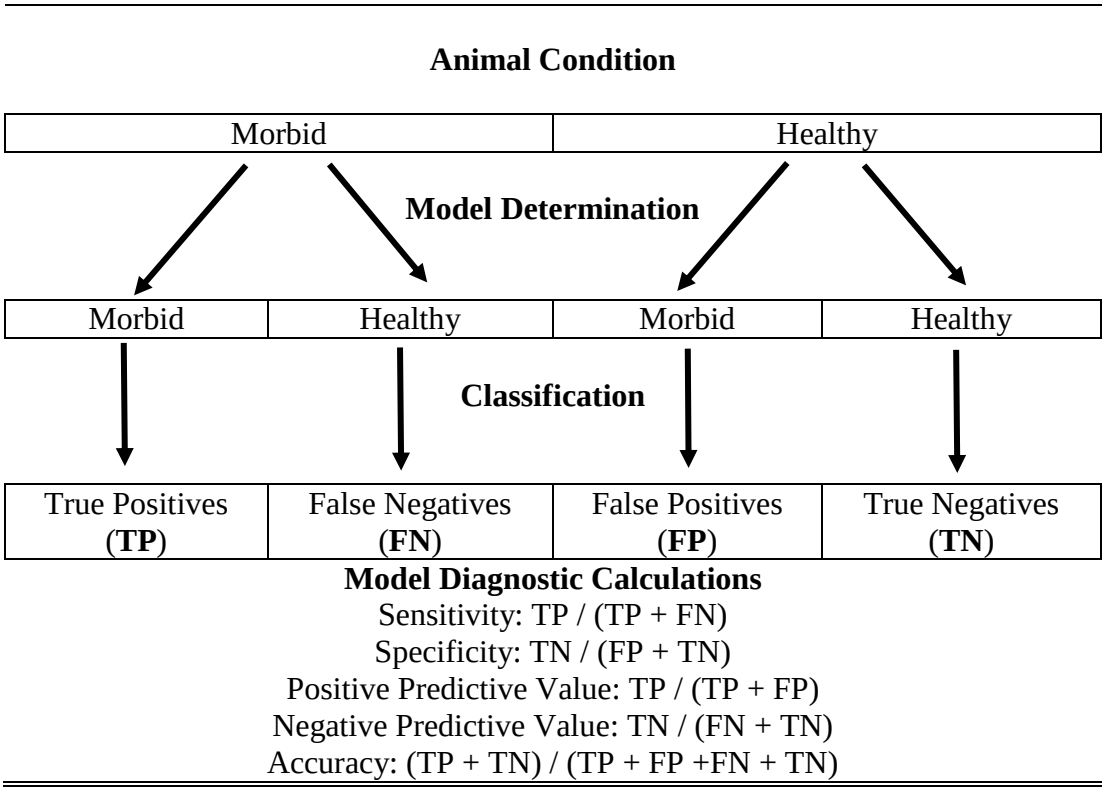

Figure 14. Diagnostic measurements and their calculations.

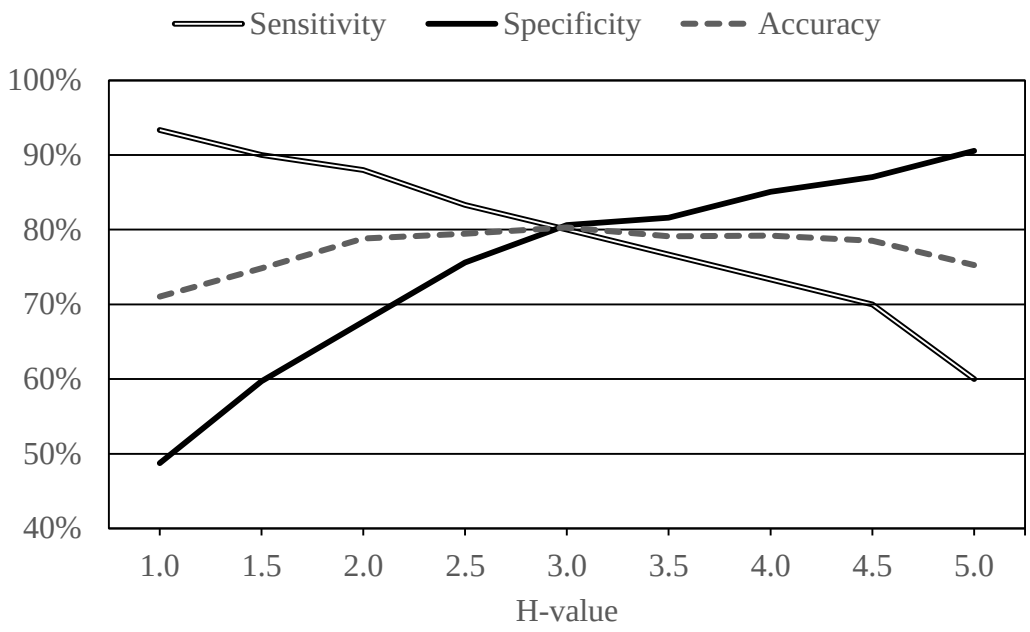

Figure 15. Effects of the H-parameter value on the sensitivity, specificity and accuracy of the CUSUM model for head down duration.

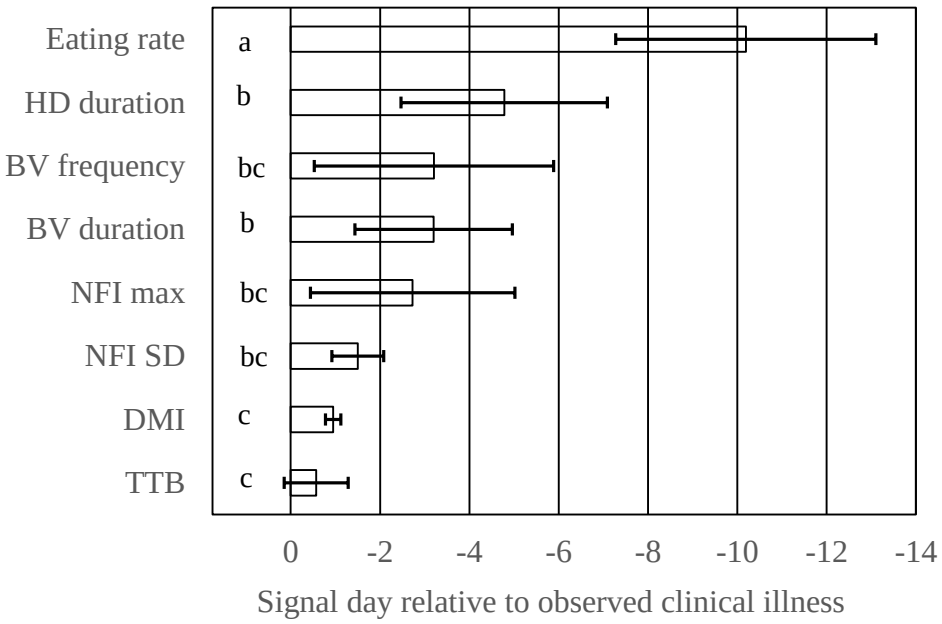

Figure 16. Mean signal day and 95% upper and lower confidence intervals for the univariate traits.
HD duration = head down duration, BV = bunk visit, NFI Max = maximal non-feeding interval per 24 hours, NFI SD = standard deviation of non-feeding interval within 24 hours. Univariate traits with unlike subscripts differ ($P < 0.05$).

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Table 13. Definitions and equations of parameters used in calculating CUSUM charts

Parameter	Equation	Description
K[1]	$K = k\sigma$	The magnitude of the deviation of an observation in SD units to be detected.
H[1]	$H = h\sigma$	The magnitude in SD units to conclude that the process is out of control. This is referred to as the decision interval.
Upper CUSUM Statistic	$C_i^+ = \max[0, y_i - K + C_{i-1}^+]$	The accumulation of deviations that are greater the than the target μ.
Lower CUSUM Statistic	$C_i^- = \min[0, K + y_i + C_{i-1}^-]$	The accumulation of deviations that are less than the target μ.

[1]Observations were standardized, therefore value of σ =1 and the parameter equations default to their selected values.

Table 14. Performance of traits in detecting morbidity, healthy animals, overall accuracy and average of days prior to clinical observations that the trait signals

Trait	Threshold	PPV	NPV	Sensitivity	Specificity	Accuracy
DMI	Lower	91.2 [b]	73.7 [c,d]	66.7 [b,c]	93.5 [b]	80.1
BV Frequency	Lower	85.4 [b]	63.3 [b,c]	46.7 [a,b,c]	92.0 [b]	69.4
BV Duration	Lower	75.3 [b]	70.1 [c]	66.7 [b,c]	78.1 [a]	72.4
HD duration	Lower	80.6 [b]	77.8 [d]	76.7 [c]	81.6 [a]	79.1
NFI Max	Upper	83.1 [b]	59.4 [a,b]	36.7 [a,b]	92.5 [b]	64.6
NFI SD	Upper	88.4 [b]	66.6 [b,c]	53.3 [a,b,c]	93.0 [b]	73.2
TTB	Upper	47.4 [a]	49.2 [a]	23.3 [a]	74.1 [a]	48.7
Eating Rate	Upper	67.3 [b]	61.4 [a,b]	53.3 [a,b,c]	74.1 [a]	63.7

PPV = positive predictive value, NPV = negative predictive value, BV = bunk visit, HD = head down, NFI Max = maximal non-feeding interval per 24 hours, NFI SD = standard deviation of non-feeding interval within 24 hours, TTB = time to bunk. Estimates within column that have unlike superscripts differ ($P < 0.05$).

Table 15. Performance of the full and reduced models along with their respective principal components in detecting morbidity, healthy animals, overall accuracy and average of days prior to clinical observations that the trait signals

Trait	Signal day	PPV	NPV	Sensitivity	Specificity	Accuracy
Full model	-2.05 [a]	77.9 [a]	72.8 [cd]	70.0 [bc]	80.1 [a]	75.0
Factor 1	-1.10 [a]	86.5 [a]	72.9 [cd]	66.7 [bc]	89.6 [abc]	78.1
Factor 2	-3.43 [a]	65.2 [a]	53.3 [a]	23.3 [a]	87.6 [abc]	55.4
RBD model	-2.08 [a]	84.4 [a]	83.5 [e]	83.3 [c]	84.6 [ab]	84.0
Factor 1	-1.23 [a]	89.7 [a]	77.4 [d]	73.3 [bc]	91.5 [bc]	82.4
Factor 2	-1.94 [a]	87.0 [a]	67.9 [bc]	56.7 [bc]	91.5 [bc]	74.1
RB model	-2.04 [a]	84.4 [a]	83.5 [e]	83.3 [c]	84.6 [ab]	84.0
Factor 1	-1.90 [a]	85.9 [a]	72.8 [cd]	66.7 [bc]	89.1 [abc]	77.9
Factor 2	-1.27 [a]	88.5 [a]	65.2 [b]	50.0 [ab]	93.5 [c]	71.8

PPV = positive predictive value, NPV = negative predictive value, RBD = reduced behavior-DMI, RB = reduced behavior. Estimates within column that have unlike superscripts differ ($P < 0.05$).

Chapter 6 Figures and Tables

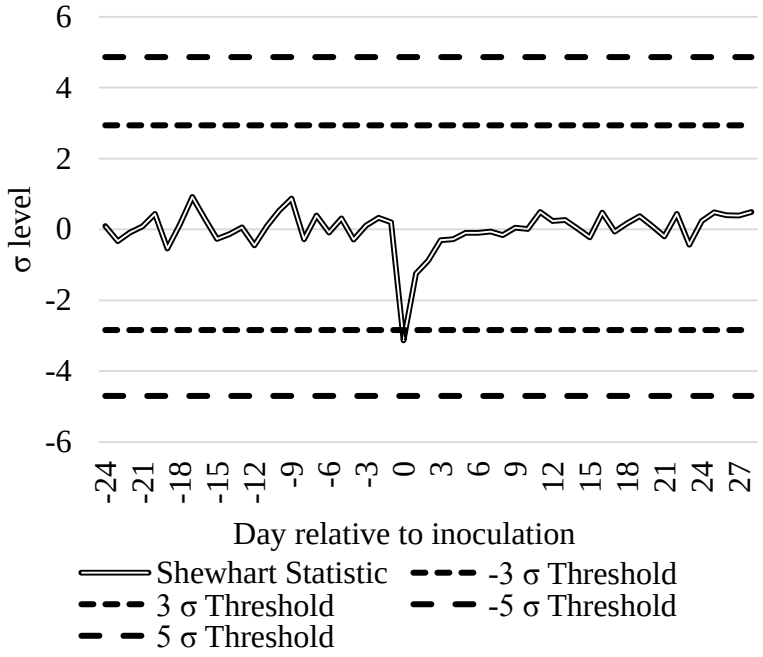

Figure 17. Shewhart chart of DMI for steers challenged with *M. haemolytica.*

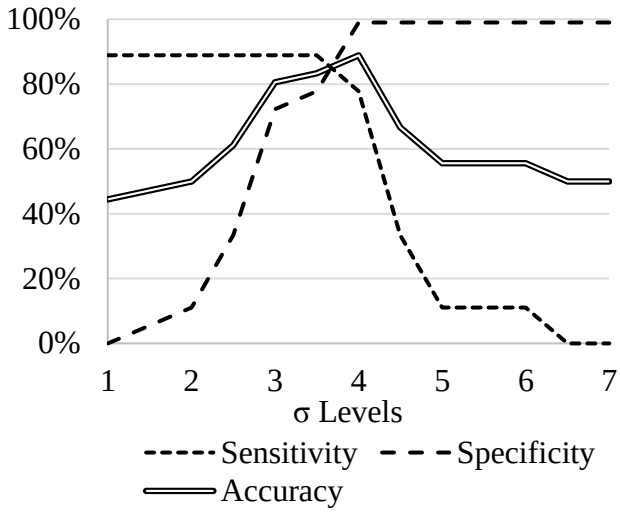

Figure 18. Sensitivity, Specificity and accuracy values for the Shewhart chart monitoring DMI at different sigma levels.

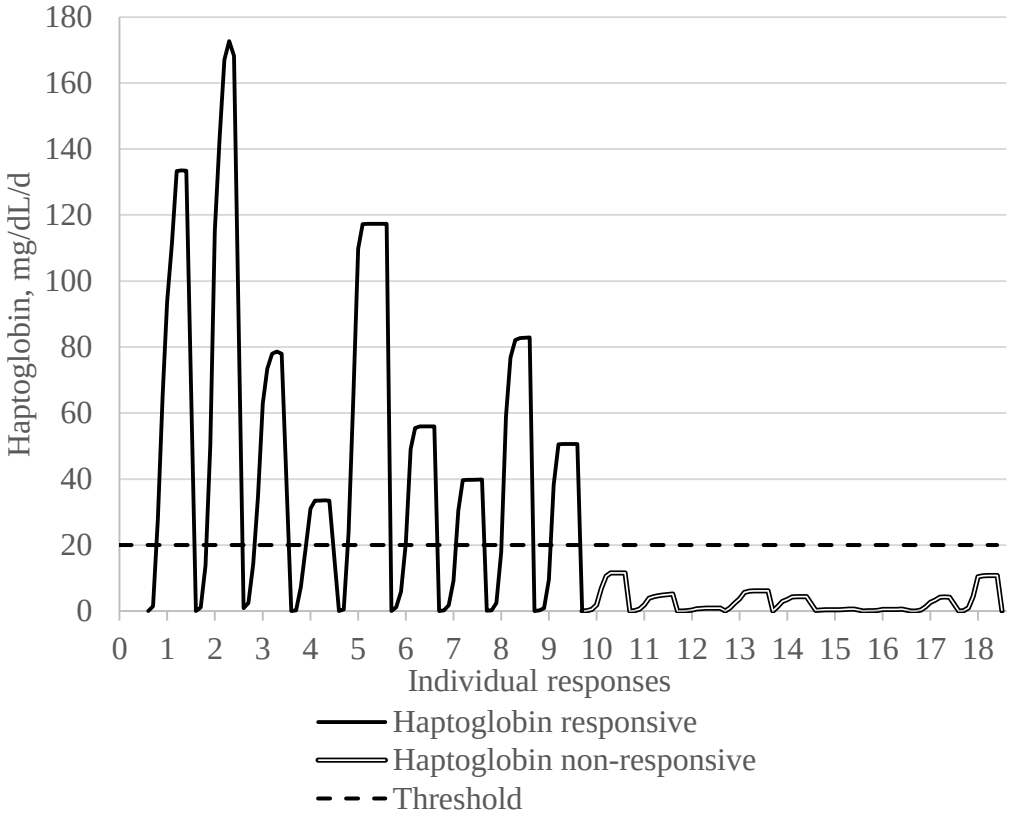

Figure 19. Area under the curve of serum concentrations of haptoglobin for individual steers that were experimentally inoculated with *M. haemolytica*.
Haptoglobin was measured from day -4 through day 14 for every animal and plotted values are presented in ascending sequential order by day for each animal.

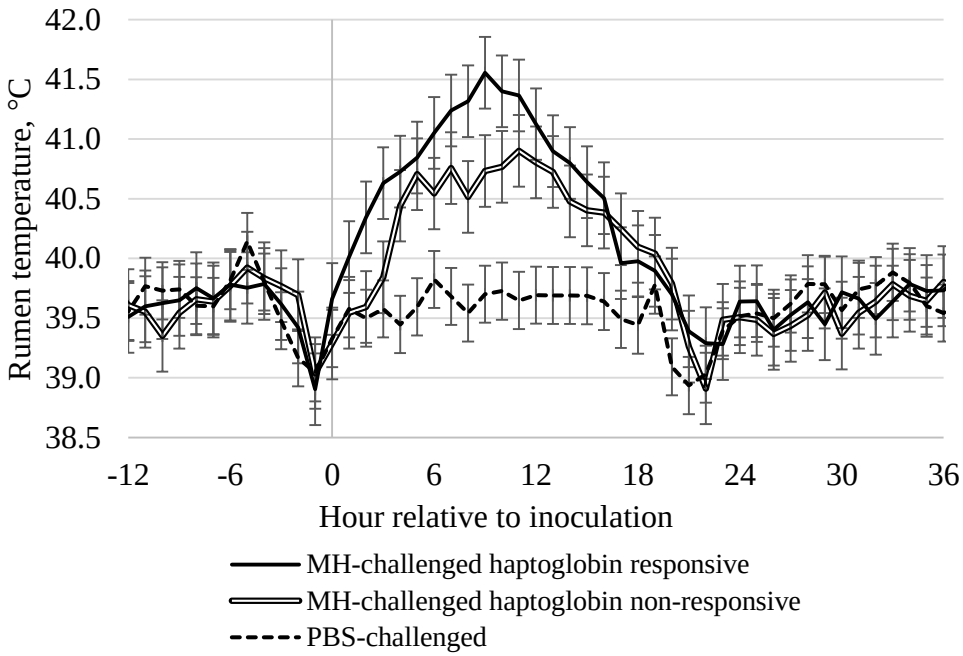

Figure 20. Rumen temperature for steers experimentally challenged with *M. haemolytica* that did and did not exhibit a haptoglobin response and steers similarly challenged with phosphate buffer solution (PBS; control).
Plotted values represent least squares mean ± 95% confidence intervals for 18 animals in the PBS group and 9 animals for the *M. haemolytica* responsive and non-responsive groups. There was a challenge by hour interaction ($P < 0.01$) and within day, values that are separated by error limits differ ($P < 0.05$).

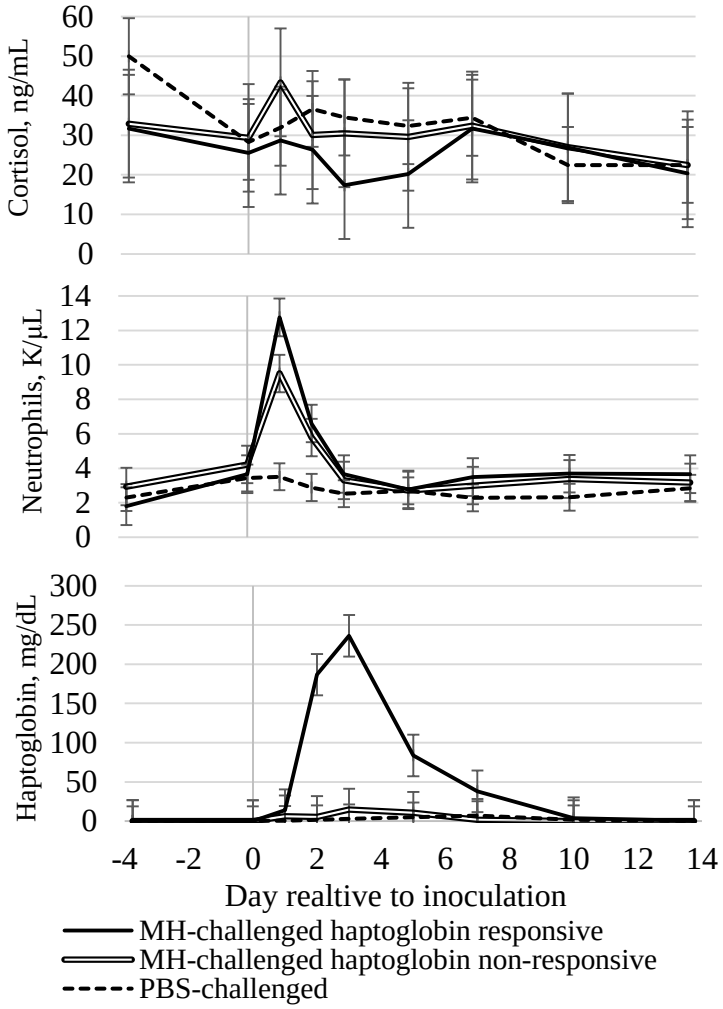

Figure 21. Serum cortisol (top), neutrophil (middle) and haptoglobin (bottom) concentrations for steers experimentally challenged with *M. haemolytica* that did and did not exhibit a haptoglobin response and steers similarly challenged with phosphate buffer solution (PBS; control).
Plotted values represent least squares mean ± 95% confidence intervals for the PBS (n = 18), *M. haemolytica* challenge haptoglobin responsive (n = 9) and non-responsive (n = 9). Challenge response type by day interaction p-values were; cortisol ($P = 0.18$), neutrophil ($P < 0.01$) and haptoglobin ($P < 0.01$) serum concentration. Within day values that are separated by error limits differ ($P < 0.05$).

Table 16. Performance of feeding behavior, accelerometer and physiologic response variables in differentiating between *M. haemolytica*-challenged and PBS-challenged steers

Effect	Sensitivity	Specificity	Accuracy	Signal Day	Signal Direction	Sigma Level
Number of Steers	18	18				
Feeding Behaviors						
DMI, kg/d	72.2^{de}	77.8^{bc}	75.0	0.08^{a}	↓	3.5
BV duration, min/d	61.1^{cde}	88.9^{bc}	75.0	0.09^{a}	↓	3.5
BV frequency, events/d	38.9^{abc}	72.2^{bc}	55.6	0.14^{a}	↓	3.0
HD duration, min/d	72.2^{de}	77.8^{bc}	75.0	0.15^{a}	↓	3.0
Time to bunk, min	94.4^{e}	5.56^{a}	50.0	0.88^{bc}	↑	3.0
Eating rate, g/min	16.7^{abc}	88.9^{bc}	52.8	4.00^{abc}	↕	4.5
Accelerometer Based Traits						
Ingestion, min/d	37.5^{abc}	82.4^{bc}	59.9	1.33^{abc}	↓	2.5
Rumination, min/d	12.5^{ab}	100^{c}	56.3	0.50^{abc}	↓	4.0
Rest, min/d	31.3^{abc}	88.2^{bc}	59.7	0.20^{a}	↕	3.0
Standing, min/d	0.00	100^{c}	50.0		↕	4.0
Over activity, min/d	0.00	94.1^{bc}	47.1		↕	4.0
Other activity, min/d	18.8^{abc}	76.5^{bc}	47.6	3.67^{abc}	↕	3.0
1st Quarter Rumen Temperature						
Average, °C	50.0^{bcde}	55.6^{b}	52.8	1.00^{b}	↑	6.5
Minimum, °C	50.0^{bcde}	72.2^{bc}	61.1	1.00^{b}	↑	5.5
Maximum, °C	44.4^{abc}	77.8^{bc}	61.1	1.00^{b}	↑	7.0
2nd Quarter Rumen Temperature						
Average, °C	38.9^{abc}	61.1^{bc}	50.0	1.00^{bc}	↑	6.0
Minimum, °C	11.1^{a}	88.9^{bc}	50.0	0.50^{abc}	↑	6.0
Maximum, °C	33.3^{abc}	66.7^{bc}	50.0	1.17^{bc}	↑	5.0
3rd Quarter Rumen Temperature						
Average, °C	50.0^{bcde}	77.8^{bc}	63.9	0.22^{a}	↑	5.5
Minimum, °C	44.4^{abc}	77.8^{bc}	61.1	0.00^{a}	↑	4.0
Maximum, °C	50.0^{bcde}	72.2^{bc}	61.1	0.22^{a}	↑	5.0
4th Quarter Rumen Temperature						
Average, °C	66.7^{de}	72.2^{bc}	69.4	0.00^{a}	↑	5.0
Minimum, °C	55.6^{bcde}	94.4^{c}	75.0	0.00^{a}	↑	5.0
Maximum, °C	61.1^{cde}	83.3^{bc}	72.2	0.00^{a}	↑	6.0

BV = bunk visit and HD = head down. Signal day is computed as the average of days post-inoculation that the effect signaled. Sigma level is the magnitude of standard deviations that the signal thresholds were most accurate. Values within column with different superscripts differ ($P < 0.05$).

Table 17. Performance of feeding behavior, accelerometer and physiologic response variables in differentiating between M. haemolytica-challenged haptoglobin responsive and PBS-challenged steers

Effect	Sensitivity	Specificity	Accuracy	Signal Day	Signal Direction	Sigma Level
Number of Steers	9	18				
Feeding Behaviors						
DMI, kg/d	77.8^{bc}	100^{c}	88.9	0.14^{a}	↓	4.0
BV duration, min/d	88.9^{bc}	88.9^{bc}	88.9	0.13^{a}	↓	3.5
BV frequency, events/d	66.7^{bc}	72.2^{bc}	69.4	0.17^{a}	↓	3.0
HD duration, min/d	88.9^{bc}	77.8^{bc}	83.3	0.25^{a}	↓	3.0
Time to bunk, min	100^{c}	5.56^{a}	52.8	1.33^{ab}	↑	3.0
Eating rate, g/min	33.3^{abc}	88.9^{bc}	61.1	4.00^{abc}	↕	4.5
Accelerometer Based Traits						
Ingestion, min/d	50.0^{bc}	82.4^{bc}	66.2	1.75^{ab}	↓	2.5
Rumination, min/d	25.0^{ab}	100^{c}	62.5	0.50^{ab}	↓	4.0
Rest, min/d	37.5^{abc}	100^{c}	68.8	0.33^{ab}	↕	4.0
Standing, min/d	0.00	100^{c}	50.0		↕	4.0
Over activity., min/d	25.0^{a}	70.6^{bc}	47.8	7.00^{c}	↕	3.0
Other activity, min/d	25.0^{ab}	76.5^{bc}	50.7	3.50^{abc}	↕	3.0
1st Quarter Rumen Temperature						
Average, °C	66.7^{bc}	44.4^{ab}	55.6	1.00^{b}	↑	5.5
Minimum, °C	44.4^{bc}	88.9^{bc}	66.7	1.00^{b}	↑	6.5
Maximum, °C	55.6^{bc}	77.8^{bc}	66.7	1.00^{b}	↑	7.0
2nd Quarter Rumen Temperature						
Average, °C	33.3^{abc}	66.7^{bc}	50.0	1.00^{ab}	↑	6.5
Minimum, °C	11.1^{ab}	94.4^{bc}	52.8	1.00	↑	7.0
Maximum, °C	33.3^{abc}	66.7^{bc}	50.0	1.30^{ab}	↑	5.5
3rd Quarter Rumen Temperature						
Average, °C	55.6^{bc}	77.8^{bc}	66.7	0.40^{ab}	↑	5.5
Minimum, °C	44.4^{bc}	77.8^{bc}	61.1	0.00^{a}	↑	4.0
Maximum, °C	55.6^{bc}	72.2^{bc}	63.9	0.40^{ab}	↑	5.0
4th Quarter Rumen Temperature						
Average, °C	77.8^{bc}	77.8^{bc}	77.8	0.00^{a}	↑	6.5
Minimum, °C	77.8^{bc}	94.4^{bc}	86.1	0.00^{a}	↑	5.0
Maximum, °C	77.8^{bc}	83.3^{bc}	80.6	0.00^{a}	↑	6.0

BV = bunk visit and HD = head down. Signal day is computed as the average of days post-inoculation that the effect signaled. Sigma level is the magnitude of standard deviations that the signal thresholds were most accurate. Values within column with different superscripts differ ($P < 0.05$).

Chapter 7 Figures and Tables

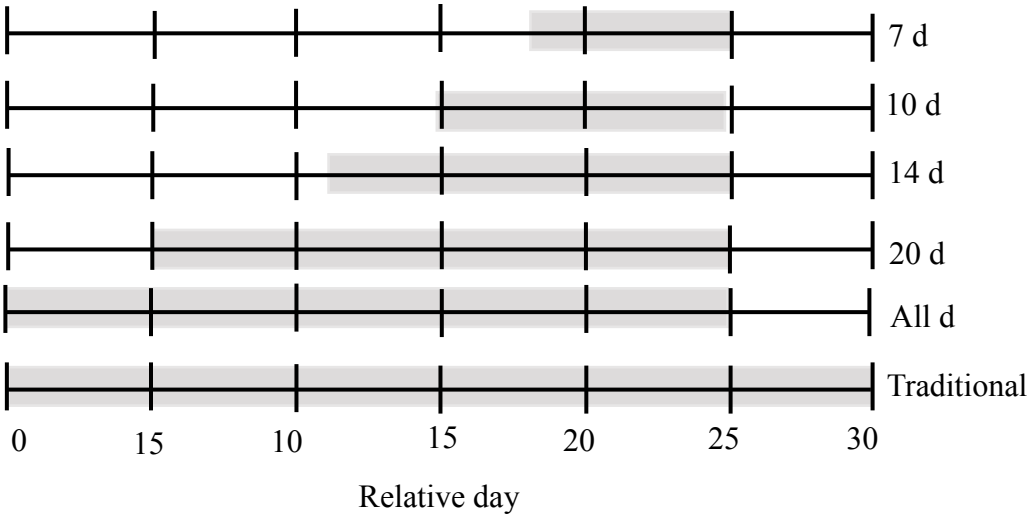

Figure 22. Representation of how the parameters were estimated using the different time windows. Gray shaded areas represent the days that are included in each parameter estimation for a given variable on day 25.

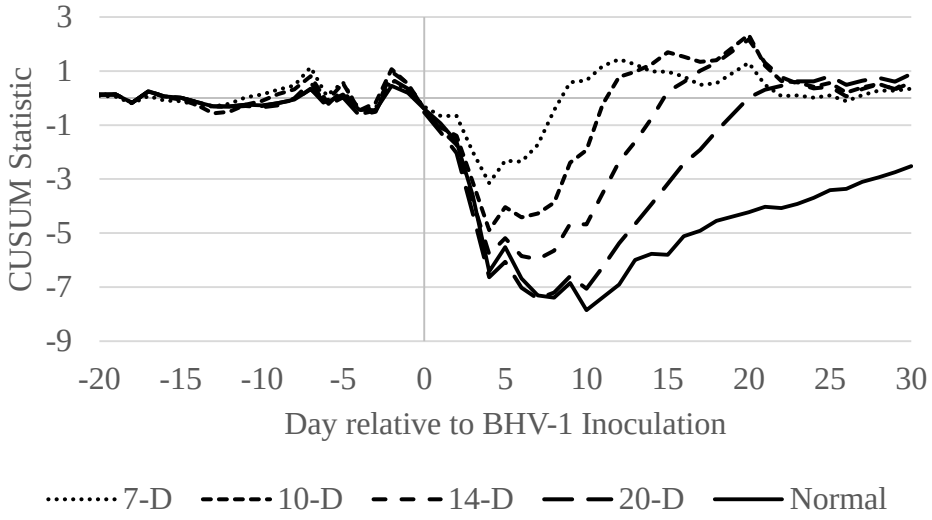

Figure 23. Average of the cumulative summation statistic (CUSUM) for DMI, calculated using the all or 7-, 10-, 14- and 20-d time windows.

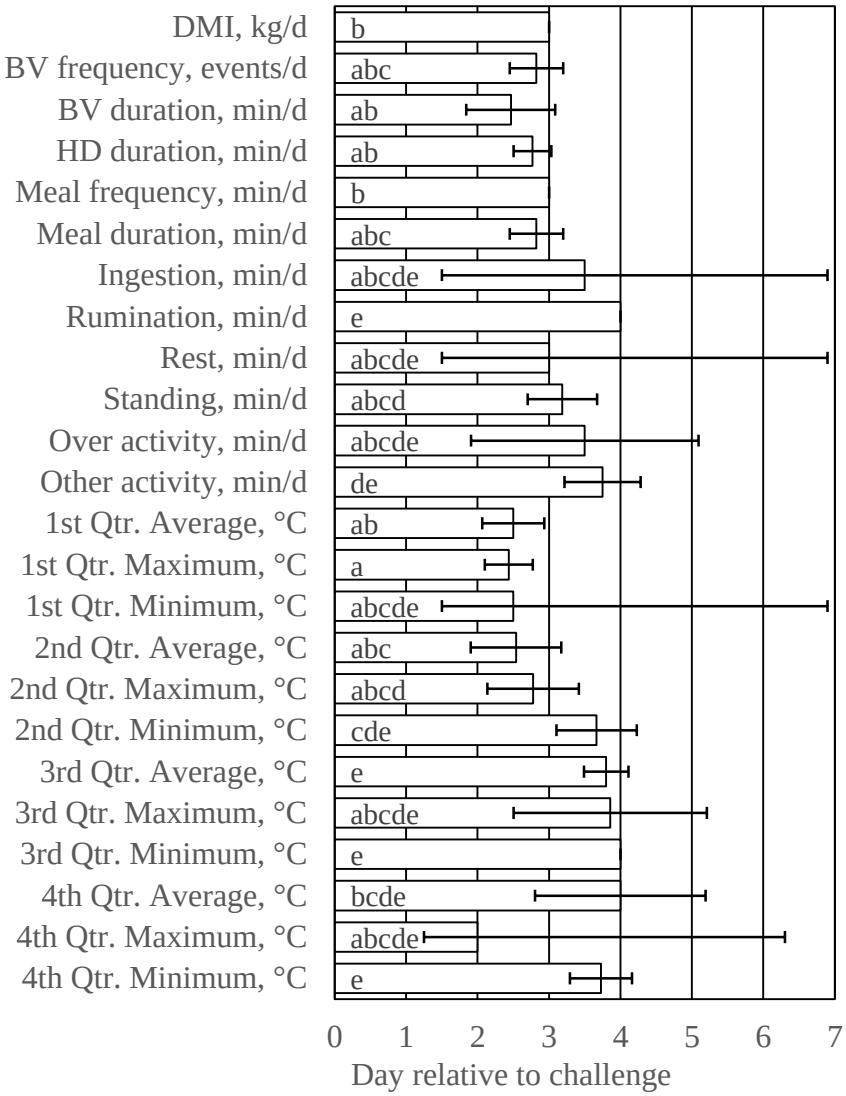

Figure 24. Shewhart model average signal day and corresponding 95% CI for each variable.
Variables with unlike superscripts differ ($P < 0.05$).

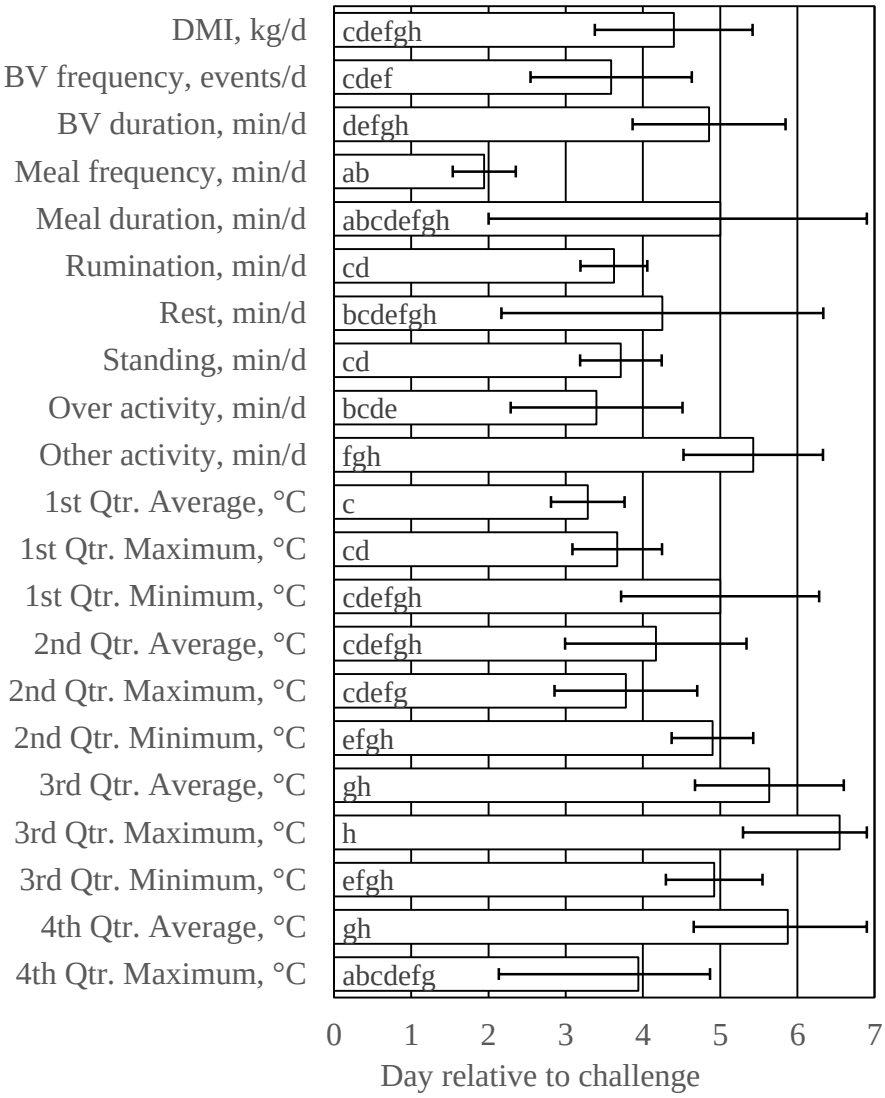

Figure 25. Cumulative summation (CUSUM) model average day and corresponding 95% CI for each variable.
Variables with unlike superscripts differ ($P < 0.05$).

Table 18. Shewhart model performance at detecting viral-bacterial challenge in heifers

Effect	Sensitivity	Specificity	Accuracy	Signal Day	Signal Direction	Sigma Level	Estimate Window
Number	19	19					
Feeding Behaviors							
DMI, kg/d	94.7_{h}	94.7_{cd}	94.7	3.0	↓	3.5	All Day
BV frequency, events/d	89.5_{gh}	73.7_{abc}	81.6	2.8	↓	4.0	All Day
BV duration, min/d	79.0_{efgh}	73.7_{abc}	76.3	2.5	↓	2.5	20-Day
HD duration, min/d	68.4_{defgh}	47.4_{ab}	57.9	2.8	↓	2.5	14-Day
Meal frequency, min/d	73.7_{defgh}	42.1_{a}	57.9	3.0	↓	2.5	20-Day
Meal duration, min/d	89.5_{gh}	84.2_{bcd}	86.8	2.8	↓	3.0	20-Day
Accelerometer Based Traits							
Ingestion, min/d	83.3_{fgh}	41.2_{a}	62.3	3.8	↓	2.5	All Day
Rumination, min/d	38.9_{abcde}	76.5_{abc}	57.7	3.9	↓	3.0	20-Day
Rest, min/d	83.3_{fgh}	94.1_{cd}	88.7	4.0	↕	3.5	20-Day
Standing, min/d	38.9_{abcde}	76.5_{abc}	57.7	4.0	↕	2.5	20-Day
Over activity, min/d	16.7_{ab}	82.4_{abcd}	49.5	2.0	↕	3.0	All Day
Other activity, min/d	61.1_{cdefg}	47.1_{ab}	54.1	3.7	↕	2.0	14-Day
1st Quarter Rumen Temperature							
Average, °C	11.1_{a}	100_{d}	55.6	3.5	↑	4.0	All Day
Maximum, °C	22.2_{abc}	94.1_{cd}	58.2	4.0	↑	3.5	All Day
Minimum, °C	16.7_{ab}	100_{d}	58.3	3.0	↑	3.5	All Day
2nd Quarter Rumen Temperature							
Average, °C	88.9_{gh}	100_{d}	94.4	3.2	↑	4.0	All Day
Maximum, °C	22.2_{abc}	100_{d}	61.1	3.5	↑	4.5	All Day
Minimum, °C	88.9_{gh}	76.5_{abc}	82.7	3.8	↑	3.0	All Day
3rd Quarter Rumen Temperature							
Average, °C	88.9_{gh}	100_{d}	94.4	2.5	↑	4.0	All Day
Maximum, °C	88.9_{gh}	100_{d}	94.4	2.4	↑	4.0	All Day
Minimum, °C	11.1_{a}	100_{d}	55.6	2.5	↑	4.5	All Day
4th Quarter Rumen Temperature							
Average, °C	72.2_{defgh}	100_{d}	86.1	2.5	↑	3.5	All Day
Maximum, °C	50.0_{bcdef}	94.1_{cd}	72.1	2.8	↑	4.0	All Day
Minimum, °C	66.7_{defgh}	82.4_{abcd}	74.5	3.7	↑	3.0	All Day

BV = bunk visit and HD = head down. Signal day is computed as the average of day post-inoculation that the variable signaled. Sigma level is the magnitude of standard deviations that the signal thresholds were most accurate. Values within column with different superscripts differ ($P < 0.05$).

Table 19. Cumulative summation (CUSUM) model performance at detecting viral-bacterial challenge in heifers

Effect	Sensitivity	Specificity	Accuracy	Signal Day	Signal Direction	K	H	Estimate Window
Number	19	19						
Feeding Behaviors								
DMI, kg/d	79.0_{cdef}	79.0_{cdef}	79.0	4.4	↓	0.75	4.0	20-Day
BV frequency, events/d	89.5_{efg}	52.6_{cd}	71.1	3.6	↓	0.75	5.5	20-Day
BV duration, min/d	36.8_{ab}	79_{cde}	57.9	4.9	↓	0.75	5.5	20-Day
HD duration, min/d	0.0	100_{f}	50.0		↓	0.75	5.0	7-Day
Meal frequency, min/d	94.7_{fg}	10.5_{ab}	52.6	1.9	↓	0.50	1.0	7-Day
Meal duration, min/d	15.8_{a}	100_{f}	57.9	5.0	↓	0.75	5.0	10-Day
Accelerometer Based Traits								
Ingestion, min/d	61.1_{bcde}	88.2_{def}	74.7	5.6	↓	0.75	4.0	All Day
Rumination, min/d	61.1_{bcde}	94.1_{ef}	77.6	6.5	↓	0.50	6.0	20-Day
Rest, min/d	72.2_{bcdef}	76.5_{cde}	74.3	4.9	↕	0.75	4.5	20-Day
Standing, min/d	44.4_{abc}	88.2_{def}	66.3	5.9	↕	0.50	3.0	All Day
Over activity, min/d	94.4_{fg}	5.9_{a}	50.2	3.9	↕	0.50	1.0	7-Day
Other activity, min/d	0.0	100_{f}	50.0		↕	0.75	4.0	7-Day
1st Quarter Rumen Temperature								
Average, °C	0.0	100_{f}	50.0		↑	0.75	5.0	7-Day
Maximum, °C	44.4_{abc}	58.8_{cd}	51.6	3.6	↑	0.75	4.5	20-Day
Minimum, °C	44.4_{abc}	70.6_{cde}	57.5	4.3	↑	0.50	6.0	20-Day
2nd Quarter Rumen Temperature								
Average, °C	77.8_{cdef}	47.1_{bc}	62.4	3.7	↑	0.75	4.5	20-Day
Maximum, °C	27.8_{ab}	64.7_{cde}	46.2	3.4	↑	0.75	6.0	14-Day
Minimum, °C	38.9_{abc}	100_{f}	69.4	5.4	↑	0.75	4.0	14-Day
3rd Quarter Rumen Temperature								
Average, °C	77.8_{cdef}	70.6_{cde}	74.2	3.3	↑	0.75	6.0	All Day
Maximum, °C	83.3_{cdef}	76.5_{cde}	79.9	3.7	↑	0.75	6.0	All Day
Minimum, °C	77.8_{cdef}	70.6_{cde}	74.2	5.0	↑	0.50	4.0	All Day
4th Quarter Rumen Temperature								
Average, °C	66.7_{bcdef}	76.5_{cde}	71.6	4.2	↑	0.75	2.5	7-Day
Maximum, °C	50.0_{abcd}	58.8_{cde}	54.4	3.8	↑	0.75	6.0	All Day
Minimum, °C	55.6_{bcde}	100_{f}	77.8	4.9	↑	0.75	5.5	All Day

BV = bunk visit and HD = head down. Signal day is computed as the average of day post-inoculation that the variable signaled. H = H value the threshold and K= K value. Values within column with different superscripts differ ($P < 0.05$).